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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (500 characters max)

METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER

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Respectfully submitted,

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Additional Page

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PROVISIONAL

PATENT APPLICATION

METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER

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METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER

FIELD OF THE INVENTION

The present invention relates to the field of biological science, more specifically to the field of cancer research. In particular, the invention relates to methods of diagnosing non-small cell lung cancers using genes, KIF11, GHSR1b, and NTSR1, that show elevated expression in such cancerous cells.

BACKGROUND OF THE INVENTION

Lung cancer is one of the most commonly fatal human tumors. Many genetic alterations associated with the development and progression of lung cancer has been reported. Although genetic changes can aid prognostic efforts and predictions of metastatic risk or response to certain treatments, information about a single or a limited number of molecular markers generally fails to provide satisfactory results for clinical diagnosis of non-small cell lung cancer (NSCLC)(Mitsudomi et al., Clin Cancer Res 6: 4055-63 (2000); Niklinski et al., Lung Cancer. 34 Suppl 2: S53-8 (2001); Watine, Bmj 320: 379-80 (2000)). NSCLC is by far the most common form, accounting for nearly 80% of lung tumors (Society, A.C. Cancer Facts and Figures 2001 (2001)). The overall 10-year survival rate remains as low as 10% despite recent advances in multi-modality therapy, because the majority of NSCLCs are not diagnosed until advanced stages (Fry, W.A., Phillips, J.L., and Menck, H.R. "Ten-year survey of lung cancer treatment and survival in hospitals in the United States: a national cancer data base report", Cancer *86:* 1867-76 (1999)). Although chemotherapy regimens based on platinum are considered the reference standards for treatment of NSCLC, those drugs are able to extend survival of patients with advanced NSCLC only about six weeks ("Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials." Non-small Cell Lung Cancer Collaborative Group, Bmj. 311: 899-909 (1995)). Numerous targeted therapies are being investigated for this disease, including tyrosine kinase inhibitors, but so far promising results have been achieved in only a limited number of patients and some recipients suffer severe adverse reactions (Kris M.N.R., Herbst R.S. "A phase II trial of ZD1839 ('Iressa') in advanced non-small cell lung cancer (NSCLC) patients who had failed platinum- and docetaxel-based regimens (IDEAL 2).", Proc. Am. Soc. Clin. Oncol. 21: 292a(A1166) (2002)).

Many genetic alterations associated with development and progression of lung cancer have been reported, but the precise molecular mechanisms remain unclear (Sozzi, G. "Molecular biology of lung cancer." Eur. J. Cancer 37: 63-73 (2001)). Over the last decade newly

developed cytotoxic agents including paclitaxel, docetaxel, gemcitabine, and vinorelbine have emerged to offer multiple therapeutic choices for patients with advanced NSCLC; however, each of the new regimens can provide only modest survival benefits compared with cisplatin-based therapies (Schiller, J.H. et al. "Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer." N. Engl. J. Med. 346: 92-98 (2002); Kelly, K. et al. "Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial." J. Clin. Oncol. 19: 3210-3218 (2001)). Hence, new therapeutic strategies, such as development of molecular-targeted agents, are eagerly awaited by clinicians.

Systematic analysis of expression levels of thousands of genes on cDNA microarrays is an effective approach to identifying unknown molecules involved in pathways of carcinogenesis (Kikuchi, T. et al. "Expression profiles of non-small cell lung cancers on cDNA microarrays: Identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs." Oncogene 22: 2192-2205 (2003); Kakiuchi, S. et al. "Genome-wide analysis of organ-preferential metastasis of human small cell lung cancer in mice." Mol. Cancer Res. 1: 485-499 (2003); Zembutsu, H. et al. "Gene-expression profiles of human tumor xenografts in nude mice treated orally with the EGFR tyrosine kinase inhibitor ZD1839." Int. J. Oncol. 23: 29-39 (2003); Suzuki, C., Daigo, Y., Kikuchi, T., Katagiri, T. & Nakamura, Y. "Identification of COX17 as a therapeutic target for non-small cell lung cancer." Cancer Res. 63: 7038-7041 (2003)) and can reveal candidate targets for development of novel anti-cancer drugs and tumor markers. To isolate novel molecular targets for diagnosis, treatment and prevention of NSCLC, the present inventors prepared have been pure populations of tumor cells were prepared from 37 cancer tissues by laser-capture microdissection and analyzed genome-wide expression profiles of NSCLC cells on a cDNA microarray containing 23,040 genes (Kikuchi, T. et al. "Expression profiles of non-small cell lung cancers on cDNA microarrays: Identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs." Oncogene 22: 2192-2205 (2003)). In the course of those experiments, KOC1 (GenBank Accession No.NM_006547) and neuromedin U (NMU; GenBank Accession No. NM_006681) were identified as genes that were frequently over-expressed in lung tumors and indispensable for growth of NSCLC cells.

The phenomenon of mRNA localization has been reported in oocytes and developing embryos of Drosophila and Xenopus and in somatic cells such as fibroblasts and neurons (King, M.L., Zhou, Y., Bubunenko, M. "Polarizing genetic information in the egg: RNA localization in the frog oocyte." Bioessays 21: 546-557 (1999); Mowry, K.L., Cote, C.A. "RNA sorting in Xenopus oocytes and embryos." FASEB J. 13: 435-445 (1999); Lasko, P. "The drosophila

melanogaster genome: translation factors and RNA binding proteins." J. Cell Biol. 150: F51-56 (2000); Steward, O. "mRNA localization in neurons: a multipurpose mechanism?" Neuron 18: 9-12 (1997)). ACTB mRNA is localized at the leading lamellae of chicken embryo fibroblasts (CEFs)(Lawrence, J.B., Singer, R.H. "Intracellular localization of messenger RNAs for cytoskeletal proteins." Cell 45: 407-415 (1986)) and at the growth cone of developing neurons (Bassell, G.J., Zhan,g H., Byrd, A.L., Femino, A.M., Singer, R.H., Taneja, K.L., Lifshitz, L.M., Herman, I.M., Kosik, K.S. "Sorting of beta-actin mRNA and protein to neurites and growth cones in culture." J. Neurosci. 18: 251-265 (1998)). The localization of ACTB mRNA is dependent of the zipcode, a cis-acting element located in the 3' UTR of the mRNA (Kislauskis, E.H., Li, Z., Singer, R.H., Taneja, K.L. "Isoform-specific 3'-untranslated sequences sort alpha-cardiac and beta-cytoplasmic actin messenger RNAs to different cytoplasmic compartments." J. Cell Biol. 123: 165-172 (1993)). The trans-acting factor, zipcode binding protein 1 (ZBP1), was affinity purified with the zipcode of ACTB mRNA (Ross, A.F., Oleynikov, Y., Kislauskis, E.H., Taneja, K.L., Singer, R.H. "Characterization of a beta-actin mRNA zipcode-binding protein." Mol. Cell Biol. 17, 2158-2165 (1997)). After the identification of ZBP1, additional homologues were identified in a wide range of organisms including Xenopus, Drosophila, human, and mouse (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." Oncogene 14: 2729-2733 (1997); Deshler, J.O., Highett, M.I., Schnapp, B.J. "Localization of Xenopus Vg1 mRNA by Vera protein and the endoplasmic reticulum." Science 276: 1128-1131 (1997); Doyle, G.A., Betz, N.A., Leeds, P.F., Fleisig, A.J., Prokipcak, R.D., Ross, J. "The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins." Nucleic Acids Res. 26: 5036-5044 (1998)). ZBP1 family members are expressed in germ embryonic fibroblasts and in several types of cancer (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." Oncogene 14: 2729-2733 (1997); Mueller, F., Bommer, M., Lacher, U., Ruhland, C., Stagge, V., Adler, G., Gress, T.M., Seufferlein, T. "KOC is a novel molecular indicator of malignancy." Br. J. Cancer 88; 699-701 (2003)). ZBP1-like proteins contain two RNA-recognition motifs (RRMs) at the NH2-terminal part of the protein and four hnRNP K homology (KH) domains at the COOH-terminal end.

KOC1 (alias IGF-II mRNA-binding protein 3: IMP-3) is one of the IMPs (IMP-1, IMP-2, and IMP-3), which belong to the ZBP1 family members and exhibit multiple attachments to

IGF-II leader 3 mRNA and the reciprocally imprinted H19 RNA (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." Oncogene 14: 2729-2733 (1997)). Although KOC1 was initially reported to be over-expressed in pancreatic cancer (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." Oncogene 14: 2729-2733 (1997); Mueller, F., Bommer, M., Lacher, U., Ruhland, C., Stagge, V., Adler, G., Gress, T.M., Seufferlein, T. "KOC is a novel molecular indicator of malignancy." Br. J. Cancer 88: 699-701 (2003)), its precise function in cancer cells or even in normal mammalian somatic cells remains unclear.

KOC1 is orthologous to the Xenopus Vg1 RNA-binding protein (Vg1RBP/Vera), which mediates the localization of Vg1 mRNA to the vegetal pole of the oocyte during oocyte maturation, and IMP-1 is orthologous to the ZBP1. IMP is mainly located at the cytoplasm and its cellular distribution ranges from a distinct concentration in perinuclear regions and lamellipodia to a completely delocalized pattern. H19 RNA co-localized with IMP, and removal of the high-affinity attachment site led to delocalization of the truncated RNA (Runge, S., Nielsen, F.C., Nielsen, J., Lykke-Andersen, J., Wewer, U.M., Christiansen, J.H. "19 RNA binds four molecules of insulin-like growth factor II mRNA-binding protein." J. Biol. Chem. 275: 29562-29569 (2000)), suggesting that IMPs are involved in cytoplasmic trafficking of RNA. IMP-1 was able to associate with microtubles (Nielsen, F.C., Nielsen, J., Kristensen, M.A., Koch, G., Christiansen, J. "Cytoplasmic trafficking of IGF-II mRNA-binding protein by conserved KH domains." J. Cell Sci. 115: 2087-2097 (2002); Havin, L., Git, A., Elisha, Z., Oberman, F., Yaniv, K., Schwartz, S.P., Standart, N., Yisraeli, J.K. "RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization." Genes Dev. 12: 1593-1598 (1998)), and is likely to involve a motor protein such as kinesin, myosin, and dyenin. On the other hand, Oskar mRNA localization to the posterior pole requires Kinesin I (Palacios, I.M., St. Johnston D. "Kinesin light chain-independent function of the Kinesin heavy chain in cytoplasmic streaming and posterior localisation in the Drosophila oocyte." Development 129: 5473-5485 (2002); Brendza, R.P., Serbus, L.R., Duffy, J.B., Saxton, W.M. "A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein." Science 289: 2120-2102 (2000)).

KIF11 (alias EG5) is a member of kinesin family, and plays a role in establishing and/or determining the stability of specific microtuble arrays that form during cell division. This role

may encompass the ability of KIF11 to influence the distribution of other protein components associated with cell division (Whitehead, C.M., Rattner, J.B. "Expanding the role of HsEg5 within the mitotic and post-mitotic phases of the cell cycle." J. Cell Sci. 111: 2551-2561 (1998); Mayer, T.U., Kapoor, T.M., Haggarty, S.J., King, R.W., Schreiber, S.L., Mitchison, T.J. "Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen." Science 286: 971-974 (1999)).

NMU is a neuropeptide that was first isolated from porcine spinal cord. It has potent activity on smooth muscles (Minamino, N., Kangawa, K. & Matsuo, H. "Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord." Biochem. Biophys. Res. Commun. 130: 1078-1085 (1985); Domin, J., Ghatei, M.A., Chohan, P. & Bloom, S.R. "Characterization of neuromedin U-like immunoreactivity in rat, porcine, guinea-pig and human tissue extracts using a specific radioimmunoassay." Biochem. Biophys. Res. Commun. 140: 1127-1134 (1986); Conlon, J.M. et al. "Primary structure of neuromedin U from the rat." J. Neurochem. 51: 988-991 (1988); Minamino, N., Kangawa, K., Honzawa, M. & Matsuo, H. "Isolation and structural determination of rat neuromedin U." Biochem. Biophys. Res. Commun. 156: 355-360 (1988); Domin, J. et al. "The distribution, purification, and pharmacological action of an amphibian neuromedin U." J. Biol. Chem. 264: 20881-20885 (1989), O'Harte, F. et al. "Primary structure and pharmacological activity of a nonapeptide related to neuromedin U isolated from chicken intestine." Peptides 12: 809-812 (1991); Kage, R., O'Harte, F., Thim, L. & Conlon, J.M. "Rabbit neuromedin U-25: lack of conservation of a posttranslational processing site." Regul. Pept. 33: 191-198 (1991); Austin, C. et al. "Distribution and developmental pattern of neuromedin U expression in the rat gastrointestinal tract." J. Mol. Endocrinol. 12: 257–263 (1994); Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." J. Biol. Chem. 275: 21068-21074 (2000)), and in mammalian species NMU is distributed predominantly in the gastrointestinal tract and central nervous system (Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." Nature 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." Peptides 23: 1607-1615 (2002)). Peripheral activities of NMU include stimulation of smooth muscle, elevation of blood pressure, alternation of ion transport in the gut, and regulation of feeding (Minamino, N., Kangawa, K. & Matsuo, H. "Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord." Biochem. Biophys. Res. Commun. 130: 1078-1085 (1985)); however, the role of NMU during carcinogenesis has not been addressed. Neuropeptides function peripherally as paracrine and autocrine factors to regulate diverse physiologic processes and act as neurotransmitters or neuromodulators in the nervous

system. In general, receptors that mediate signaling by binding neuropeptides are members of the superfamily of G protein-coupled receptors (GPCRs) having seven transmembrane-spanning domains. Two known receptors for NMU, NMU1R and NMU2R, show a high degree of homology to other neuropeptide receptors such as GHSR and NTSR1, for which the corresponding known ligands are Ghrelin (GHRL) and neurotensin (NTS), respectively. NMU1R (FM3/GPR66) and NMU2R (FM4) have seven predicted alpha-helical transmembrane domains containing highly conserved motifs, as do other members of the rhodopsin GPCR family (Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." J. Biol. Chem. 275: 21068-21074 (2000); Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." Nature 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." Peptides 23: 1607-1615 (2002)).

A C-terminal asparaginamide structure and the C-terminal hepatapeptide core of NMU protein are essential for its contractile activity in smooth-muscle cells (Westfall, T.D. et al. "Characterization of neuromedin U effects in canine smooth muscle." J. Pharmacol. Exp. Ther. 301: 987-992 (2002); Austin, C., Nandha, K.A., Meleagros, L. & Bloom, S.R. "Cloning and characterization of the cDNA encoding the human neuromedin U precursor: NMU expression in the human gastrointenstinal tract." J. Mol. Endocrinol. 14: 157-169 (1995)). Recent studies have contributed evidence that NMU acts at the hypothalamic level to inhibit food intake; therefore this protein might be a physiological regulator of feeding and body weight (Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." Nature 406: 70-74 (2000); Maggi, C.A. et al. "Motor response of the human isolated small intestine and urinary bladder to porcine neuromedin U-8." Br. J. Pharmacol. 99: 186-188 (1990); Wren, A.M. et al. "Hypothalamic actions of neuromedin U." Endocrinology 143: 227-234 (2002); Ivanov, T.R., Lawrence, C.B., Stanley, P.J. & Luckman, S.M. "Evaluation of neuromedin U actions in energy homeostasis and pituitary function." Endocrinology 143: 3813-3821 (2002)). However, so far no reports have suggested involvement of NMU over-expression in carcinogenesis.

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnesyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45 (1999)). Clinical trials on human using a combination or anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of

breast-cancer patients (Lin et al., Cancer Res. 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita et al., Cancer Res. 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, Int. J. Cancer 54: 177-80 (1993); Boon and van der Bruggen, J. Exp. Med. 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J. Exp. Med. 178: 489-95 (1993); Kawakami et al., J. Exp. Med. 180: 347-52 (1994)). Some of the discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., Science 254: 1643-7 (1991)), gp100 (Kawakami et al., J. Exp. Med. 180: 347-52 (1994)), SART (Shichijo et al., J. Exp. Med. 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., Proc. Natl. Acad. Sci. USA 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano et al., Brit. J. Cancer 84: 1052-7 (2001)), HER2/neu (Tanaka et al., Brit. J. Cancer 84: 94-9 (2001)), CEA (Nukaya et al., Int. J. Cancer 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., Nature Med. 4: 321-7 (1998); Mukherji et al., Proc. Natl. Acad. Sci. USA 92: 8078-82 (1995); Hu et al., Cancer Res. 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, J. Exp. Med. 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J. Exp. Med. 178: 489-95 (1993); Kawakami et al.,J. Exp. Med. 180: 347-52 (1994); Shichijo et al., J. Exp. Med. 187: 277-88 (1998); Chen et al., Proc. Natl. Acad. Sci. USA 94: 1914-8 (1997); Harris, J. Natl. Cancer Inst. 88: 1442-5 (1996);

Butterfield et al., Cancer Res. 59: 3134-42 (1999); Vissers et al., Cancer Res. 59: 5554-9 (1999); van der Burg et al., J Immunol 156: 3308-14 (1996); Tanaka et al., Cancer Res. 57: 4465-8 (1997); Fujie et al., Int. J. Cancer 80: 169-72 (1999); Kikuchi et al., Int. J. Cancer 81: 459-66 (1999); Oiso et al., Int. J. Cancer 81: 387-94 (1999)).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or -A0201 restricted manner in ^{51}Cr-release assays (Kawano et al., Cancer Res. 60: 3550-8 (2000); Nishizaka et al., Cancer Res. 60: 4830-7 (2000); Tamura et al., Jpn. J. Cancer Res. 92: 762-7 (2001)). However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101 (1996); Kondo et al., J. Immunol. 155: 4307-12 (1995); Kubo et al., J. Immunol. 152: 3913-24 (1994); Imanishi et al., Proceeding of the eleventh International Histocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams et al., Tissue Antigen 49: 129 (1997)). Thus, antigenic peptides of cancers presented by these HLAs may be especially useful for the treatment of cancers among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL *in vitro* usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc. Natl. Acad. Sci. USA 93: 4102-7 (1996)).

Although advances have been made in the development of molecular-targeting drugs for cancer therapy, the ranges of tumor types that respond as well as the effectiveness of the treatments are still very limited. Hence, it is urgent to develop new anti-cancer agents that target molecules highly specific to malignant cells and are likely to cause minimal or no adverse reactions. To achieve the goal molecules whose physiological mechanisms are well defined need to be identified. A powerful strategy toward these ends would combine screening of up-regulated genes in cancer cells on the basis of genetic information obtained on cDNA microarrays with high-throughput screening of their effect on cell growth, by inducing loss-of-function phenotypes with RNAi systems (Kikuchi, T., Daigo, Y., Katagiri, T., Tsunoda, T., Okada, K., Kakiuchi, S., Zembutsu, H., Furukawa, Y., Kawamura, M., Kobayashi, K., Imai, K., Nakamura, Y. "Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs", Oncogene 22: 2192-2205 (2003)).

SUMMARY OF THE INVENTION

The present invention features a method of diagnosing or determining a predisposition to non-small cell lung cancer (NSCLC) in a subject by determining an expression level of a non-small cell lung cancer-associated gene that is selected from the group of KIF11, GHSR1b, and NTSR1 in a patient derived biological sample. An increase of the expression level of any of the genes compared to a normal control level of the genes indicates that the subject suffers from or is at risk of developing NSCLC.

A "normal control level" indicates an expression level of any of the genes detected in a normal, healthy individual or in a population of individuals known not to be suffering from NSCLC. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. In contrast to a "normal control level", the "control level" is an expression level of the gene detected in an individual or a population of individuals whose background of the disease state is known (i.e., cancerous or non-cancerous). Thus, the control level may be determined base on the expression level of the gene in a normal, healthy individual, in a population of individuals known not to be suffering from NSCLC, a patient of NSCLC or a population of the patients. The control level corresponding to the expression level of the gene in a patient of non-small cell lung cancer or a population of the patients is referred to as "NSCLC control level". Furthermore, the control level can be a database of expression patterns from previously tested cells.

An increase in the expression level of any one of the genes of KIF11, GHSR1b, and NTSR1 detected in a test biological sample compared to a normal control level indicates that the subject (from which the sample was obtained) suffers from NSCLC. Alternatively, the expression level of any one or all of the genes in a biological sample may be compared to an NSCLC control level of the same gene(s).

Gene expression is increased or decreased 10%, 25%, 50% or more compared to the control level. Alternatively, gene expression is increased or decreased 1, 2, 5 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on a chip or an array, of an NSCLC gene probe to a gene transcript of a patient-derived biological sample. The patient-derived biological sample may be any sample derived from a subject, *e.g.*, a patient known to or suspected of having NSCLC. For example, the biological sample may be tissue containing sputum, blood, serum, plasma or lung cell.

The invention also provides a non-small cell lung cancer reference expression profile comprising a pattern of gene expression levels of two or more genes selected from the group of KIF11, GHSR1b, and NTSR1.

The invention also provides a kit comprising two or more detection reagents which detects the expression of one or more of genes selected from the group of KIF11, GHSR1b, and NTSR1 (e.g., via detecting mRNA and polypeptide). Also provided is an array of polynucleotides that binds to one or more of the genes selected from the group of KIF11, GHSR1b, and NTSR1.

The invention further provides methods of identifying compounds that inhibit the expression level of an NSCLC-associated gene (KIF11, GHSR1b, or NTSR1) by contacting a test cell expressing an NSCLC-associated gene with a test compound and determining the expression level of the NSCLC-associated gene. The test cell may be an NSCLC cell. A decrease of the expression level compared to a normal control level of the gene indicates that the test compound is an inhibitor of the expression or function of the NSCLC-associated gene. Therefore, if a compound suppresses the expression level of KIF11, GHSR1b, or NTSR1 compared to a normal control level, the compound is expected to reduce a symptom of NSCLC.

Alternatively, the present invention provides a method of screening for a compound for treating or preventing NSCLC. The method includes contacting a polypeptide selected from the group of KIF11, GHSR1b and NTSR1 with a test compound, and selecting the test compound that binds to or suppresses the biological activity of the polypeptide. The invention further provides a method of screening for a compound for treating or preventing NSCLC, which includes the steps of contacting a test compound with a cell that expresses KIF11, GHSR1b or NTSR1 protein or introduced with a vector comprising the transcriptional regulatory region of KIF11, GHSR1b or NTSR1 gene upstream of a reporter gene, and then selecting the test compound that reduces the expression level of the KIF11, GHSR1b or NTSR1 protein or protein encoded by the reporter gene. According to these screening methods, the test compound that suppresses the biological activity or the expression level compared to a normal control level is expected to reduce a symptom of NSCLC. Furthermore, the present invention provides a method of screening for a compound for treating or preventing NSCLC wherein the binding between KIF11 and KOC1, or GHSR1b or NTSR1 and NMU is detected. Compounds that inhibit the binding between KIF11 and KOC1, or GHSR1b or NTSR1 and NMU are expected to reduce a symptom of NSCLC.

Methods for treating or preventing NSCLC and compositions to be used for such methods are also provided. Therapeutic methods include a method of treating or preventing NSCLC in a subject by administering to the subject a composition of an antisense, short interfering RNA (siRNA) or a ribozyme that reduce the expression of KIF11, GHSR1b or NTSR1 gene, or a composition comprising an antibody or fragment thereof that binds and suppresses the function

of a polypeptide encoded by the gene.

The invention also includes vaccines and vaccination methods. For example, a method of treating or preventing NSCLC in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by KIF11, GHSR1b or NTSR1 gene, or an immunologically active fragment of the polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein and which induces an immune response upon introduction into the body. For example, an immunologically active fragment includes a polypeptide of at least 8 residues in length that stimulates an immune cell such as a T cell or a B cell *in vivo*. Immune cell stimulation can be measured by detecting cell proliferation, elaboration of cytokines (*e.g.*, IL-2) or production of antibody.

Other therapeutic methods include those wherein a compound selected by the screening method of the present invention is administered.

Also included in the invention are double-stranded molecules that comprise a sense strand and an antisense strand. The sense strand comprises a ribonucleotide sequence corresponding to a target sequence comprised within the mRNA of a KIF11, GHSR1b or NTSR1 gene, and the antisense strand is a complementary sequence to the sense strand. Such double-stranded molecules of the present invention can be used as siRNAs against KIF11, GHSR1b or NTSR1 gene. Furthermore, the present invention relates to vectors encoding the double-stranded molecules of the present invention.

The present application also provides a composition for treating and/or preventing NSCLC using any of the antisense polynucleotides or siRNAs against KIF11, GHSR1b or NTSR1 gene, or an antibody that binds to a polypeptide encoded by KIF11, GHSR1b or NTSR1 gene. Other compositions include those that contain a compound selected by the screening method of the present invention as an active ingredient.

It is to be understood that both the foregoing summary of the invention and the following detailed description are of a preferred embodiment, and not restrictive of the invention or other alternate embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a photograph depicting the result of SDS-PAGE identifying the KOC1-interacting protein. The protein complex including KOC1 was purified from LC319 cells that were transfected with bilateral-tagged (pCAGGS-n3FH vector: N-terminal; Flag, C-terminal;

HA) KOC1-expressing vector by immunoprecipitation (IP) (two step IP: 1st IP by anti-Flag agarose, followed by the 2nd IP by anti-HA antibody). As a control, mock- and other gene-construct-transfected LC319 cells were prepared. Immunoprecipitated products were stained by silver staining on the SDS-PAGE gel. A 120 kDa band was extracted and determined to be KIF11 by Mass spectrometric sequencing (in the figure, indicated with an arrow head).

Fig. 2 shows photographs confirming the relationship between KOC1 and KIF11. Fig. 2A depicts the result of co-immunoprecipitation of KOC1 and KIF11 confirming the interaction between KOC1 and KIF11. A549 cells were transiently co-transfected with Flag-tagged KIF11 and myc-tagged KOC1, immunoprecipitated with anti-Flag M2 agarose, and subsequently immunoblotted with anti-myc antibody. In contrast, using the same combination of vectors and cells, the cells were immunoprecipitated with anti-myc agarose and immunoblotted with anti-Flag M2 antibody. A band corresponding to the immunoblotted protein was found only when both constructs were co-transfected. Fig. 2B depicts the result of immunocytochemical staining showing the co-localization of KOC1 and KIF11. COS-7 cells were transiently transfected with FLAG-tagged KIF11 and myc-tagged KOC1, and their co-localization was detected mainly in the cytoplasm using FITC-labeled anti-FLAG antibody and rhomamine-labeled anti-myc antibody.

Fig. 3 shows the result of semiquantitative RT-PCR demonstrating the expression of KOC1 and KIF11 in clinical samples of NSCLC, corresponding normal lung tissues, and NSCLC cell lines. Similar mRNA expression pattern was found between KIF11 and KOC1 in NSCLC.

Fig. 4 shows KIF11 expression in normal human tissues. Similar mRNA expression pattern was found between KIF11 and KOC1 in normal human tissues.

Fig.5 shows the effect of siRNAs on cells. Fig. 5A depicts the inhibition on the growth of NSCLC cells by siRNAs against KIF11. The expression of KIF11 in response to specific siRNAs (si-KIF#1, #2, #3, #4, and #5) or control siRNAs (EGFP, LUC, SC) in A549 cells, was analyzed by semiquantitative RT-PCR. Fig. 5B depicts the viability of A549 cells in response to si-KIF#1, #2, #3, #4, #5, EGFP, LUC, or SC, evaluated by triplicate MTT assays.

Fig. 6 shows structural and functional characteristics of KIF11. Fig. 6A is a schematic illustration of KIF11. KIF11 is a member of the BimC family of kinesin related proteins whose members are characterized by a conserved, globular motor domain at the

N-terminus followed by a non-conserved, rod-like helical coiled-coil domain and a BimC box at the C-terminus. Fig. 6B depicts a schematic model mechanism of mRNA localization by KOC1 and KIF11.

Fig. 7 shows the result of semiquantitative RT-PCR analysis depicting the expression of NMU, candidate receptors, and their known ligands detected in NSCLC cell lines.

Fig. 8 shows the relationship between NMU and GHSR1b/NTSR1. Fig. 8A depicts the result of immunocytochemical staining using FITC-labeled anti-FLAG antibody showing the co-localization of NMU and GHSR1b/NTSR1 on the cell surface of COS-7 cells that were transiently transfected with FLAG-tagged GHSR1b or NTSR1. Fig. 8B depicts the interaction of NMU with GHSR1b/NTSR1. COS-7 cells were transiently transfected with the same vectors, and binding of rhodamine-labeled NMU-25 to the cell surface was detected by flow cytometry. As negative controls for these assays, three ligand/cell combinations were prepared: 1) non-transfected COS-7 cells; 2) NMU-25-rhodamine vs non-transfected COS-7 cells; and 3) COS-7 cells transfected only with GHSR1b or NTSR1.

Fig. 9 shows GHSR1b expression in normal human tissues.

Fig. 10 shows the effect of siRNAs on cells. Fig. 10A depicts the inhibition on the growth of NSCLC cells by siRNAs against GHSR1b and NTSR1. Expression of GHSR or NTSR1 in response to specific siRNAs (si-GHSR or si-NTSR1) or control siRNAs (EGFP, LUC, SCR) in A549 and LC319 cells were analyzed by semiquantitative RT-PCR. Fig. 10B depicts the result of triplicate MTT assays evaluating viability of A549 or LC319 cells in response to si-GHSR, NTSR1, EGFP, LUC, or SCR.

Fig. 11 is a schematic illustration of structural and functional characteristics of GHSR1b and NTSR1.

DETAILED DESCRIPTION OF THE INVENTION

The words "a", "an" and "the" as used herein mean "at least one" unless otherwise specifically indicated. The terms "protein" and "polypeptide" are used interchangeably. Furthermore, the terms "gene", "polynucleotide", and "nucleic acids" are used interchangeably unless otherwise specifically indicated.

To investigate the mechanisms of lung carcinogenesis and identify genes that might be useful as diagnostic markers or targets for development of new molecular therapies, genes specifically

up-regulated in non-small cell lung cancers (NSCLC) were searched by means of cDNA microarray. Through the analysis, a couple of candidate therapeutic target genes were identified. Two genes among them, KH domain containing protein over-expressed in cancer (KOC1) and neuromedin U (NMU) were abundantly expressed in clinical NSCLC samples as well as NSCLC cell lines examined. However, their expressions were hardly detectable in corresponding non-cancerous lung tissue. The growth of NSCLC cells that over-expressed endogenous NMU was significantly inhibited by anti-NMU antibody. Furthermore, the treatment of NSCLC cells with siRNA against KOC1 or NMU suppressed the expression of the gene and resulted in growth inhibition of the NSCLC cells. Furthermore, KOC1 was identified to bind to kinesin family member 11 (KIF11) of the cancer cells, whereas NMU bound to the neuropeptide G protein-coupled receptors (GPCRs), growth hormone secretagogue receptor 1b (GHSR1b) and neurotensin receptor 1 (NTSR1). Interestingly, GHSR1b, NTSR1, and KIF11 were all specifically over-expressed in NSCLC cells.

RNA binding protein KOC1 and microtubles motor protein KIF11 may be required for the localization of some kinds of mRNA needed in embryogenesis and carcinogenesis (Fig. 6). As previously reported by the present inventors, treatment of NSCLC cells with specific siRNA to reduce expression of KOC1 resulted in growth suppression. In this study, KIF11 was demonstrated to associate with KOC1 in NSCLC cells and to be the likely target for the growth-promoting effect of KOC1 in lung tumors. The present inventors revealed that KOC1 not only co-localized with KIF11 in human normal tissues, NSCLCs, and cell lines, but also directly interacted with KIF11 in NSCLC cells *in vitro*, and that the treatment of NSCLC cells with siRNAs for KIF11 reduced its expression and led to growth suppression. The results suggest that KOC1-KIF11 signaling affects growth of NSCLC cells. By expression analysis, increased expression of KOC1 and KIF11 was detected in the majority of NSCLC samples, but not in normal lung tissues. Since most of the clinical NSCLC samples used for the present analysis were at an early and operable stage, KOC1 and KIF11 might serve as a biomarker for diagnosing early-stage lung cancer, in combination with fiberscopic transbronchial biopsy (TBB) or sputum cytology.

Therefore, KOC1 and KIF11 are likely to be essential for an oncogenic pathway in NSCLCs. The data reported here strongly imply the possibility of designing new anti-cancer drugs, specific for lung cancer, which target the KOC1-KIF11 pathway. They also suggest a potential for siRNAs themselves to interfere with this pathway, as a novel approach to treatment of chemotherapy-resistant, advanced lung cancers.

A significant increase in the sub-G1 fraction of NSCLC cells transfected with siRNA-NMU

suggested that blocking the autocrine NMU-signaling pathway could induce apoptosis. The present inventors also found other evidence supporting the significance of this pathway in carcinogenesis; e.g., addition of NMU into the medium promoted the growth of COS-7 cells in a dose-dependent manner, and addition of anti-NMU antibody into the culture medium inhibited this NMU-enhanced cell growth, possibly by neutralizing NMU activity. Moreover, the growth of NSCLC cells that endogenously over-expressed NMU was significantly inhibited by anti-NMU antibody. The expression of NMU also resulted in significant promotion of COS-7 cell invasion in *in vitro* assays. These results strongly suggested that NMU is likely to be an important growth factor for NSCLC and might be associated with cancer cell invasion, functioning in an autocrine manner, and that screening molecules targeting the NMU-receptor growth-promoting pathway should be a promising therapeutic approach for treating NSCLCs. By immunohistochemical analysis, increased expression of NMU protein was detected in the majority of NSCLC (SCC, ADC, LCC, and BAC) and SCLC samples, but not in normal lung tissues. Since NMU is a secreted protein and most of the clinical NSCLC samples used for the present analysis were at an early and operable stage, NMU might serve as a biomarker for diagnosis of early-stage lung cancer, in combination with fiberscopic transbronchial biopsy (TBB), sputum cytology, or blood tests.

Two receptors, NMU1R (FM3/GPR66) and NMU2R (FM4) are known to interact with NMU. The results presented here, however, indicated that these two known receptors were not the targets for the autocrine NMU-signaling pathway in NSCLCs; instead, GHSR1b and NTSR1 proved to be the likely targets for the growth-promoting effect of NMU in lung tumors. The present inventors revealed that NMU-25 bound to these receptors on the cell surface, and that treatment of NSCLC cells with siRNAs for GHSR1b or NTSR1 reduced expression of the receptors and led to apoptosis. The results suggest that NMU affects growth of NSCLC cells by acting through GHSR1b and/or NTSR1 (Fig. 11). GHSR is a known receptor of Ghrelin (GHRL), a recently identified 28-amino-acid peptide capable of stimulating release of pituitary growth hormone and appetite in humans (Lambert, P.D. et al. "Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity." Proc. Natl. Acad. Sci. 98: 4652-4657 (2001); Petersenn, S., Rasch, A.C., Penshorn, M., Beil, F.U. & Schulte, H.M. "Genomic structure and transcriptional regulation of the human growth hormone secretagogue receptor." Endocrinology 142: 2649-2659 (2001); Kim K. et al. "Ghrelin and growth hormone (GH) secretagogue receptor (GHSR) mRNA expression in human pituitary adenomas." Clin. Endocrinol. 54: 705-860 (2001); Kojima, M. et al. "Ghrelin is a growth-hormone-releasing acylated peptide from stomach." Nature 402: 656-660 (1999)). Of the two transcripts known to be receptors for

GHRL, GHSR1a and GHSR1b, over-expression of only GHSR1b was detected in NSCLC tissues and cell lines. Since GHRL was not expressed in the NSCLCs examined, GHSR1b was suspected to have a growth-promoting function in lung tumors through binding to NMU, but not to GHRL.

NTSR1 is one of three receptors of neurotensin (NTS), a brain and gastrointestinal peptide that fulfills many central and peripheral functions (Heasley, L.E. "Autocrine and paracrine signaling through neuropeptide receptors in human cancer." Oncogene 20: 1563-1569 (2001)). NTS modulates transmission of dopamine and secretion of pituitary hormones, and exerts hypothermic and analgesic effects in the brain while it functions as a peripheral hormone in the digestive tract and cardiovascular system. Others have reported that NTS is produced and secreted in several human cancers, including small-cell lung cancers (SCLC) (Heasley, L.E. "Autocrine and paracrine signaling through neuropeptide receptors in human cancer." Oncogene 20: 1563-1569 (2001)). The expression of NTS was detected in four of the 15 NSCLC cell lines that were examined in the present invention (Fig. 7), but the expression pattern of NTS was not necessarily concordant with that of NMU or NTSR1. Therefore NTS may, along with NMU, contribute to the growth of NSCLC through NTSR1 or other receptor(s) in a small subset of NSCLCs. In the present experiments the majority of the cancer cell lines and clinical NSCLCs that expressed NMU also expressed GHSR1b and/or NTSR1, indicating that these ligand-receptor interactions were likely to be involved in a pathway that is central to the growth-promoting activity of NMU in NSCLCs.

Therefore, NMU and two newly revealed receptors for this molecule, GHSR1b and NTSR1, are likely to be essential for an autocrine growth-promoting pathway in NSCLCs. The data reported here strongly imply the possibility of designing new anti-cancer drugs, specific for lung cancer, that target the NMU-GHSR1b/NTSR1 pathway. They also suggest a potential for siRNAs themselves to interfere with this pathway, as a novel approach to treatment of chemotherapy-resistant, advanced lung cancers.

These data strongly suggest that KOC1-KIF11 signaling pathway is frequently up-regulated in lung carcinogenesis, and that NMU is likely to be an important autocrine growth factor for NSCLC, acting through GHSR1b and NTSR1 receptor molecules. Thus, selective suppression of components of these complexes may function to suppress the development and/or progression of lung carcinogenesis and targeting these pathways might provide promising therapeutic and diagnostic strategies for the treatment of lung-cancer patients.

Diagnosing non-small cell lung cancer (NSCLC)

By measuring the expression level of KIF11, GHSR1b or NTSR1 gene in a biological derived from a subject, the occurrence of NSCLC or a predisposition to develop NSCLC in the subject can be determined. The invention involves determining (*e.g.*, measuring) the expression level of at least one, and up to all of KIF11, GHSR1b and NTSR1 gene in the biological sample.

According to the present invention, a gene transcript of NSCLC-associated gene, KIF11, GHSR1b or NTSR1, is detected for determining the expression level of the gene. The expression level of a gene can be detected by detecting the expression products of the gene, including both transcriptional and translational products, such as mRNA and proteins. Based on the sequence information provided by the GenBank™ database entries for the known sequences, KIF11, GHSR1b and NTSR1 genes can be detected and measured using techniques well known to one of ordinary skill in the art. The nucleotide sequences of the KIF11, GHSR1b and NTSR1 genes are described as SEQ ID NOs: 1, 3 and 5, respectively, and the amino acid sequences of the proteins encoded by the genes are described as SEQ ID NOs: 2, 4 and 6.

For example, sequences within the sequence database entries corresponding to KIF11, GHSR1b or NTSR1 gene can be used to construct probes for detecting their mRNAs by, *e.g.*, Northern blot hybridization analysis. The hybridization of the probe to a gene transcript in a subject biological sample can be also carried out on a DNA array. The use of an array is preferred for detecting the expression level of a plurality of the NSC genes (KIF11, GHSR1b and NTSR1). As another example, the sequences can be used to construct primers for specifically amplifying KIF11, GHSR1b or NTSR1 gene in, *e.g.*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction (RT-PCR). Furthermore, the expression level of KIF11, GHSR1b or NTSR1 gene can be analyzed based on the quantity of the expressed proteins encoded by the gene. A method for determining the quantity of the expressed protein includes immunoassay methods. Alternatively, the expression level of KIF11, GHSR1b or NTSR1 gene can also be determined based on the biological activity of the expressed protein encoded by the gene. For example, a protein encoded by KIF11 gene is known to bind to KOC1, and thus the expression level of the gene can be detected by measuring the binding ability to KOC1 due to the expressed protein. Furthermore, KIF11 protein is known to have a cell proliferating activity. Therefore, the expression level of KIF11 gene can be determined using such cell proliferating activity as an index. On the other hand GHSR1b and NTSR1 proteins are known to bind to NMU, and also have a cell proliferating activity. Thus,

similarly to KIF11, the expression levels of GHSR1b and NTSR1 genes can be detected by measuring their binding ability to NMU or cell proliferating activity due to the expressed protein.

Any biological materials may be used as the biological sample for determining the expression level so long as any of the KIF11, GHSR1b and NTSR1 genes can be detected in the sample and includes test cell populations (*i.e.*, subject derived tissue sample). Preferably, the biological sample comprises a lung cell (a cell obtained from the lung). Gene expression may also be measured in blood, serum or other bodily fluids such as sputum. Furthermore, the test sample may be cells purified from a tissue.

The subject diagnosed for NSCLC according to the method is preferably a mammal and includes human, non-human primate, mouse, rat, dog, cat, horse and cow.

The expression level of one or more of KIF11, GHSR1b or NTSR1 gene in the biological sample is compared to the expression level(s) of the same genes in a reference sample. The reference sample includes one or more cells with known parameters, *i.e.*, cancerous or non-cancerous. The reference sample should be derived from a tissue type similar to that of the test sample. Alternatively, the control expression level may be determined based on a database of molecular information derived from cells for which the assayed parameter or condition is known.

Whether or not a pattern of the gene expression levels in a biological sample indicates the presence of NSCLC depends upon the composition of the reference cell population. For example, when the reference cell population is composed of non-cancerous cells, a similar gene expression level in the test biological sample to that of the reference indicates that the test biological sample is non-cancerous. On the other hand, when the reference cell population is composed of cancerous cells, a similar gene expression profile in the biological sample to that of the reference indicates that the test biological sample includes cancerous cells.

The test biological sample may be compared to multiple reference samples. Each of the multiple reference samples may differ in the known parameter. Thus, a test sample may be compared to a reference sample known to contain, *e.g.*, NSCLC cells, and at the same time to a second reference sample known to contain, *e.g.*, non-NSCLC cells (normal cells).

According to the invention, the expression of one or more of the NSCLC-associated genes, KIF11, GHSR1b and NTSR1, is determined in the biological sample and compared to the normal control level of the same gene. The phrase "normal control level" refers to an expression profile of KIF11, GHSR1b or NTSR1 gene typically found in a biological sample derived from a population not suffering from NSCLC. The expression level of KIF11,

GHSR1b or NTSR1 gene in the biological samples from a control and test subjects may be determined at the same time or the normal control level may be determined by a statistical method based on the results obtained by analyzing the expression level of the gene in samples previously collected from a control group. An increase of the expression level of KIF11, GHSR1b or NTSR1 gene in the biological sample derived from a patient derived tissue sample indicates that the subject is suffering from or is at risk of developing NSCLC.

An expression level of KIF11, GHSR1b or NTSR1 gene in a test biological sample can be considered altered when the expression level differs from that of the reference by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold. Alternatively, an expression level of KIF11, GHSR1b or NTSR1 gene in a test biological sample can be considered altered, when the expression level is increased or decreased to that of the reference at least 50%, 60%, 80%, 90% or more.

The difference in gene expression between the test sample and a reference sample may be normalized to a control, *e.g.*, housekeeping gene. For example, a control polynucleotide includes those whose expression levels are known not to differ between the cancerous and non-cancerous cells. The expression levels of the control polynucleotide in the test and reference samples can be used to normalize the expression levels detected for KIF11, GHSR1b or NTSR1 gene. The control genes to be used in the present invention include β-actin, glyceraldehyde 3-phosphate dehydrogenase and ribosomal protein P1.

The differentially expressed KIF11, GHSR1b or NTSR1 gene identified herein also allow for monitoring the course of treatment of NSCLC. In this method, a test biological sample is provided from a subject undergoing treatment for NSCLC. If desired, multiple test biological samples are obtained from the subject at various time points before, during or after the treatment. The expression of one or more of KIF11, GHSR1b or NTSR1 gene in the sample is then determined and compared to a reference sample with a known state of NSCLC that has not been exposed to the treatment.

If the reference sample contains no NSCLC cells, a similarity in the expression level of KIF11, GHSR1b or NTSR1 gene in the test biological sample and the reference sample indicates the efficaciousness of the treatment. However, a difference in the expression level of KIF11, GHSR1b or NTSR1 gene in the test and the reference samples indicates a less favorable clinical outcome or prognosis.

The term "efficacious" refers that the treatment leads to a reduction in the expression of a pathologically up-regulated gene (including the present indicator genes, KIF11, GHSR1b and NTSR1), or a decrease in size, prevalence or metastatic potential of NSCLC in a subject.

When a treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents occurrence of NSCLC or alleviates a clinical symptom of NSCLC. The assessment of NSCLC can be made using standard clinical protocols. Furthermore, the efficaciousness of a treatment is determined in association with any known method for diagnosing or treating NSCLC. For example, NSCLC is diagnosed histopathologically or by identifying symptomatic anomalies such as chronic cough, hoarseness, coughing up blood, weight loss, loss of appetite, shortness of breath, wheezing, repeated bouts of bronchitis or pneumonia and chest pain.

Moreover, the present method for diagnosing NSCLC may also be applied for assessing the prognosis of a patient with the cancer by comparing the expression level of KIF11, GHSR1b or NTSR1 gene in the patient-derived biological sample. Alternatively, the expression level of the gene(s) in the biological sample may be measured over a spectrum of disease stages to assess the prognosis of the patient.

An increase in the expression level of KIF11, GHSR1b or NTSR1 gene compared to a normal control level indicates less favorable prognosis. A similarity in the expression level of KIF11, GHSR1b or NTSR1 gene compared to a normal control level indicates a more favorable prognosis of the patient. Preferably, the prognosis of a subject can be assessed by comparing the expression profile of KIF11, GHSR1b or NTSR1 gene.

Expression profile

The invention also provides an NSCLC reference expression profile comprising a pattern of gene expression levels of two or more of KIF11, GHSR1b and NTSR1 genes. The expression profile serves as a control for the diagnosis of NSCLC or predisposition for developing the disease, monitoring the course of treatment and assessing prognosis of a subject with the disease.

Kit

The invention also provides a kit comprising two or more detection reagents, *e.g.*, a nucleic acid that specifically binds to or identifies one or more of KIF11, GHSR1b and NTSR1 genes. Such nucleic acids specifically binding to or identifying one or more of KIF11, GHSR1b and NTSR1 genes are exemplified by oligonucleotide sequences that are complementary to a portion of KIF11, GHSR1b or NTSR1 polynucleotides or antibodies which bind to polypeptides encoded by the KIF11, GHSR1b or NTSR1gene. The reagents are packaged together in the form of a kit. The reagents, such as a nucleic acid or antibody (either bound to a solid matrix

or packaged separately with reagents for binding them to the matrix), a control reagent (positive and/or negative) and/or a means of detection of the nucleic acid or antibody are preferably packaged in separate containers. Instructions (*e.g.*, written, tape, VCR, CD-ROM, etc.) for carrying out the assay may be included in the kit. The assay format of the kit may be Northern hybridization or sandwich ELISA known in the art.

For example, a detection reagent is immobilized on a solid matrix such as a porous strip to form at least one detection site. The measurement or detection region of the porous strip may include a plurality of detection sites, each detection site containing a detection reagent. A test strip may also contain sites for negative and/or positive controls. Alternatively, control site(s) is located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized reagents, *i.e.*, a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of a test biological sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of KIF11, GHSR1b or NTSR1 gene, or polypeptides encoded by the gene present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

Alternatively, the kit contains a nucleic acid substrate array comprising two or more of the KIF11, GHSR1b and NTSR1 gene sequences. The expression of 2 or 3 of the genes represented by KIF11, GHSR1b and NTSR1 genes are identified by virtue of the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a solid substrate, *e.g.*, a "chip" as described in U.S. Patent No. 5,744,305.

Array and pluralities

The invention also includes a nucleic acid substrate array comprising one or more of the KIF11, GHSR1b and NTSR1 genes. The nucleic acids on the array specifically correspond to one or more polynucleotide sequences represented by KIF11, GHSR1b and NTSR1 genes. The expression level of 2 or 3 of the KIF11, GHSR1b and NTSR1 genes is identified by detecting the binding of nucleic acid to the array.

The invention also includes an isolated plurality (*i.e.*, a mixture of two or more nucleic acids) of nucleic acids. The nucleic acids are in a liquid phase or a solid phase, *e.g.*, immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the polynucleotides represented by KIF11, GHSR1b and NTSR1 genes. According to a further embodiment of the present invention, the plurality includes 2 or 3 of the polynucleotides

represented by KIF11, GHSR1b and NTSR1 genes.

Chips

The DNA chip is a device that is convenient to compare the expression levels of a number of genes at the same time. DNA chip-based expression profiling can be carried out, for example, by the method as disclosed in "Microarray Biochip Technology " (Mark Schena, Eaton Publishing, 2000), etc.

A DNA chip comprises immobilized high-density probes to detect a number of genes. Thus, the expression levels of many genes can be estimated at the same time by a single-round analysis. Namely, the expression profile of a specimen can be determined with a DNA chip. The DNA chip-based method of the present invention comprises the following steps of:

(1) synthesizing aRNAs or cDNAs corresponding to the marker genes;

(2) hybridizing the aRNAs or cDNAs with probes for marker genes; and

(3) detecting the aRNA or cDNA hybridizing with the probes and quantifying the amount of mRNA thereof.

The aRNA refers to RNA transcribed from a template cDNA with RNA polymerase. An aRNA transcription kit for DNA chip-based expression profiling is commercially available. With such a kit, aRNA can be synthesized from T7 promoter-attached cDNA as a template using T7 RNA polymerase. On the other hand, by PCR using random primer, cDNA can be amplified using as a template a cDNA synthesized from mRNA.

Alternatively, the DNA chip comprises probes, which have been spotted thereon, to detect the marker genes of the present invention (KIF11, GHSR1b or NTSR1 gene). There is no limitation on the number of marker genes spotted on the DNA chip, and 1, 2 or all of the genes, KIF11, GHSR1b and NTSR1, may be used. Any other genes as well as the marker genes can be spotted on the DNA chip. For example, a probe for a gene whose expression level is hardly altered may be spotted on the DNA chip. Such a gene can be used to normalize assay results when the assay results are intended to be compared between multiple chips or between different assays.

A probe is designed for each marker gene selected, and spotted on a DNA chip. Such a probe may be, for example, an oligonucleotide comprising 5-50 nucleotide residues. A method for synthesizing such oligonucleotides on a DNA chip is known to those skilled in the

art. Longer DNAs can be synthesized by PCR or chemically. A method for spotting long DNA, which is synthesized by PCR or the like, onto a glass slide is also known to those skilled in the art. A DNA chip that is obtained by the method as described above can be used for diagnosing NSCLC according to the present invention.

The prepared DNA chip is contacted with aRNA, followed by the detection of hybridization between the probe and aRNA. The aRNA can be previously labeled with a fluorescent dye. A fluorescent dye such as Cy3(red) and Cy5 (green) can be used to label an aRNA. aRNAs from a subject and a control are labeled with different fluorescent dyes, respectively. The difference in the expression level between the two can be estimated based on a difference in the signal intensity. The signal of fluorescent dye on the DNA chip can be detected by a scanner and analyzed using a special program. For example, the Suite from Affymetrix is a software package for DNA chip analysis.

Identifying compounds that inhibit NSCLC-associated gene expression

A compound that inhibits the expression or activity of a target NSCLC-associated gene (KIF11, GHSR1b or NTSR1 gene) is identified by contacting a test cell expressing the NSCLC-associated gene with a test compound and determining the expression level or activity of the NSCLC-associated gene. A decrease in expression compared to the normal control level indicates that the compound is an inhibitor of the NSCLC-associated gene. Such compounds identified according to the method are useful for inhibiting NSCLC.

The test cell may be a population of cells and includes any cells as long as the cell expresses the target NSCLC-associated gene(s). For example, the test cell may be an immortalized cell line derived from an NSCLC cell. Alternatively, the test cell may be a cell transfected with any of the KIF11, GHSR1b and NTSR1 genes, or which has been transfected with the regulatory sequence (*e.g.*, promoter) of any of the genes that is operably linked to a reporter gene.

Screening compounds

Using KIF11, GHSR1b or NTSR1 gene, proteins encoded by the gene or transcriptional regulatory region of the gene, compounds can be screened that alter the expression of the gene or biological activity of a polypeptide encoded by the gene. Such compounds are expected to serve as pharmaceuticals for treating or preventing NSCLC.

Therefore, the present invention provides a method of screening for a compound for treating or preventing NSCLC using the polypeptide of the present invention. An embodiment of this screening method comprises the steps of: (a) contacting a test compound with a polypeptide encoded by KIF11, GHSR1b or NTSR1 gene; (b) detecting the binding activity between the polypeptide of the present invention and the test compound; and (c) selecting the compound that binds to the polypeptide.

The polypeptide to be used for the screening may be a recombinant polypeptide or a protein derived from the nature or a partial peptide thereof. The polypeptide to be contacted with a test compound can be, for example, a purified polypeptide, a soluble protein, a form bound to a carrier or a fusion protein fused with other polypeptides.

As a method of screening for proteins that bind to KIF11, GHSR1b or NTSR1 polypeptide, many methods well known by a person skilled in the art can be used. Such a screening can be conducted by, for example, immunoprecipitation method, specifically, in the following manner. A gene encoding any of the KIF11, GHSR1b and NTSR1 polypeptides is expressed in animal cells and so on by inserting the gene into an expression vector for foreign genes, such as pSV2neo, pcDNA I, pcDNA3.1, pCAGGS and pCD8. The promoter to be used for the expression may be any promoter that can be used commonly and include, for example, the SV40 early promoter (Rigby in Williamson (ed.), Genetic Engineering, vol. 3. Academic Press, London, 83-141 (1982)), the EF-α promoter (Kim et al., Gene 91: 217-23 (1990)), the CAG promoter (Niwa et al., Gene 108: 193-200 (1991)), the RSV LTR promoter (Cullen, Methods in Enzymology 152: 684-704 (1987)) the SRα promoter (Takebe et al., Mol Cell Biol 8: 466 (1988)), the CMV immediate early promoter (Seed and Aruffo, Proc Natl Acad Sci USA 84: 3365-9 (1987)), the SV40 late promoter (Gheysen and Fiers, J Mol Appl Genet 1: 385-94 (1982)), the Adenovirus late promoter (Kaufman et al., Mol Cell Biol 9: 946 (1989)), the HSV TK promoter and so on. The introduction of the gene into animal cells to express a foreign gene can be performed according to any methods, for example, the electroporation method (Chu et al., Nucleic Acids Res 15: 1311-26 (1987)), the calcium phosphate method (Chen and Okayama, Mol Cell Biol 7: 2745-52 (1987)), the DEAE dextran method (Lopata et al., Nucleic Acids Res 12: 5707-17 (1984); Sussman and Milman, Mol Cell Biol 4: 1642-3 (1985)), the Lipofectin method (Derijard, B Cell 7: 1025-37 (1994); Lamb et al., Nature Genetics 5: 22-30 (1993): Rabindran et al., Science 259: 230-4 (1993)), and so on. The NSC polypeptide can also be expressed as a fusion protein comprising a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the monoclonal antibody, whose specificity has been revealed, to the N- or C- terminus of the polypeptide. A commercially available epitope-antibody system can be used (Experimental Medicine 13: 85-90 (1995)). Vectors

which can express a fusion protein with, for example, β-galactosidase, maltose binding protein, glutathione S-transferase, green florescence protein (GFP), and so on, by the use of its multiple cloning sites are commercially available.

A fusion protein prepared by introducing only small epitopes consisting of several to a dozen amino acids so as not to change the property of the original polypeptide by the fusion is also reported. Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage) and such, and monoclonal antibodies recognizing them can be used as the epitope-antibody system for screening proteins binding to the KIF11, GHSR1b or NTSR1 polypeptide (Experimental Medicine 13: 85-90 (1995)).

In immunoprecipitation, an immune complex is formed by adding these antibodies to cell lysate prepared using an appropriate detergent. The immune complex consists of the KIF11, GHSR1b or NTSR1 polypeptide, a polypeptide comprising the binding ability with the polypeptide, and an antibody. Immunoprecipitation can be also conducted using antibodies against the KIF11, GHSR1b or NTSR1 polypeptide, in addition to the use of antibodies against the above epitopes, which antibodies can be prepared according to conventional methods and may be in any form, such as monoclonal or polyclonal antibodies, and includes antiserum obtained by immunizing an animal such as a rabbit with the polypeptide, all classes of polyclonal and monoclonal antibodies, as well as recombinant antibodies (e.g., humanized antibodies).

Herein, an antibody is defined as a protein that reacts with either the full length or a fragment of KIF11, GHSR1b or NTSR1 polypeptide. Specifically, antibodies against KIF11, GHSR1b or NTSR1 polypeptide can be prepared as follows. KIF11, GHSR1b or NTSR1 polypeptide used as an antigen to obtain an antibody may be derived from any animal species, but preferably is derived from a mammal such as a human, mouse, or rat, more preferably from a human. According to the present invention, the polypeptide to be used as an immunization antigen may be a complete protein or a partial peptide of the KIF11, GHSR1b or NTSR1 polypeptide. A partial peptide may comprise, for example, the amino (N)-terminal or carboxy (C)-terminal fragment of the KIF11, GHSR1b or NTSR1 polypeptide.

A gene encoding KIF11, GHSR1b or NTSR1 polypeptide, or its fragment may be inserted into a known expression vector, which is then used to transform a host cell. The desired protein or its fragment may be recovered from the outside or inside of host cells by any standard method, and may subsequently be used as an antigen. Alternatively, whole cells

expressing the KIF11, GHSR1b or NTSR1 polypeptide or their lysates, or a chemically synthesized polypeptide may be used as the antigen.

Any mammalian animal may be immunized with the antigen, but preferably the compatibility with parental cells used for cell fusion is taken into account. In general, animals of Rodentia, Lagomorpha or Primates are used. Animals of Rodentia include, for example, mouse, rat and hamster. Animals of Lagomorpha include, for example, rabbit. Animals of Primates include, for example, a monkey of Catarrhini (old world monkey) such as *Macaca fascicularis*, rhesus monkey, sacred baboon and chimpanzees.

Methods for immunizing animals with antigens are known in the art. Intraperitoneal injection or subcutaneous injection of antigens is a standard method for immunization of mammals. More specifically, antigens may be diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, etc. If desired, the antigen suspension may be mixed with an appropriate amount of a standard adjuvant, such as Freund's complete adjuvant, made into emulsion, and then administered to mammalian animals. Preferably, it is followed by several administrations of the antigen mixed with an appropriately amount of Freund's incomplete adjuvant every 4 to 21 days. An appropriate carrier may also be used for immunization. After immunization as above, the serum is examined by a standard method for an increase in the amount of desired antibodies.

Polyclonal antibodies against KIF11, GHSR1b or NTSR1 polypeptide may be prepared by collecting blood from the immunized mammal examined for the increase of desired antibodies in the serum, and by separating serum from the blood by any conventional method. Polyclonal antibodies include serum containing the polyclonal antibodies, as well as the fraction containing the polyclonal antibodies may be isolated from the serum. Immunoglobulin G or M can be prepared from a fraction which recognizes only the KIF11, GHSR1b or NTSR1 polypeptide using, for example, an affinity column coupled with the polypeptide, and further purifying this fraction using protein A or protein G column.

To prepare monoclonal antibodies, immune cells are collected from the mammal immunized with the antigen and checked for the increased level of desired antibodies in the serum as described above, and are subjected to cell fusion. The immune cells used for cell fusion are preferably obtained from spleen. Other preferred parental cells to be fused with the above immunocyte include, for example, myeloma cells of mammalians, and more preferably myeloma cells having an acquired property for the selection of fused cells by drugs.

The above immunocyte and myeloma cells can be fused according to known methods, for

example, the method of Milstein et al. (Galfre and Milstein, Methods Enzymol 73: 3-46 (1981)).

Resulting hybridomas obtained by the cell fusion may be selected by cultivating them in a standard selection medium, such as HAT medium (hypoxanthine, aminopterin, and thymidine containing medium). The cell culture is typically continued in the HAT medium for several days to several weeks, the time being sufficient to allow all the other cells, with the exception of the desired hybridoma (non-fused cells), to die. Then, the standard limiting dilution is performed to screen and clone a hybridoma cell producing the desired antibody.

In addition to the above method, in which a non-human animal is immunized with an antigen for preparing hybridoma, human lymphocytes such as those infected by EB virus may be immunized with KIF11, GHSR1b or NTSR1 polypeptide, cells expressing the polypeptide, or their lysates *in vitro*. Then, the immunized lymphocytes are fused with human-derived myeloma cells that are capable of indefinitely dividing, such as U266, to yield a hybridoma producing a desired human antibody that is able to bind to the KIF11, GHSR1b or NTSR1 polypeptide can be obtained (Unexamined Published Japanese Patent Application No. (JP-A) Sho 63-17688).

The obtained hybridomas are subsequently transplanted into the abdominal cavity of a mouse and the ascites are extracted. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column, DEAE ion exchange chromatography, or an affinity column to which any of the target proteins of the present invention (KIF11, GHSR1b and NTSR1 polypeptide) is coupled. The antibody can be used not only in the present screening method, but also for purification and detection of KIF11, GHSR1b or NTSR1 polypeptide, and serve also as candidates for agonists and antagonists of the polypeptide. In addition, this antibody can be applied to the antibody treatment for diseases related to the KIF11, GHSR1b or NTSR1 polypeptide including NSCLC as described *infra*.

Monoclonal antibodies thus obtained can be also recombinantly prepared using genetic engineering techniques (see, for example, Borrebaeck and Larrick, Therapeutic Monoclonal Antibodies, published in the United Kingdom by MacMillan Publishers LTD (1990)). For example, a DNA encoding an antibody may be cloned from an immune cell, such as a hybridoma or an immunized lymphocyte producing the antibody, inserted into an appropriate vector, and introduced into host cells to prepare a recombinant antibody. Such recombinant antibody can also be used for the present screening.

Furthermore, an antibody used in the screening and so on may be a fragment of an antibody or modified antibody, so long as it binds to one or more of KIF11, GHSR1b and NTSR1 polypeptides. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston et al., Proc Natl Acad Sci USA 85: 5879-83 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co et al., J Immunol 152: 2968-76 (1994); Better and Horwitz, Methods Enzymol 178: 476-96 (1989); Pluckthun and Skerra, Methods Enzymol 178: 497-515 (1989); Lamoyi, Methods Enzymol 121: 652-63 (1986); Rousseaux et al., Methods Enzymol 121: 663-9 (1986); Bird and Walker, Trends Biotechnol 9: 132-7 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). Modified antibodies can be obtained through chemically modification of an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from nonhuman antibody and the constant region derived from human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from nonhuman antibody, the frame work region (FR) derived from human antibody, and the constant region. Such antibodies can be prepared using known technology.

Antibodies obtained as above may be purified to homogeneity. For example, the separation and purification of the antibody can be performed according to separation and purification methods used for general proteins. For example, the antibody may be separated and isolated by the appropriately selected and combined use of column chromatographies, such as affinity chromatography, filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis, isoelectric focusing, and others (Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but are not limited thereto. A protein A column and protein G column can be used as the affinity column. Exemplary protein A columns to be used include, for example, Hyper D, POROS, and Sepharose F.F. (Pharmacia).

Exemplary chromatography, with the exception of affinity includes, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, adsorption chromatography, and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring

Harbor Laboratory Press (1996)). The chromatographic procedures can be carried out by liquid-phase chromatography, such as HPLC and FPLC.

An immune complex can be precipitated, for example with Protein A sepharose or Protein G sepharose when the antibody is a mouse IgG antibody. If the KIF11, GHSR1b or NTSR1 polypeptide is prepared as a fusion protein with an epitope, such as GST, an immune complex can be formed in the same manner as in the use of the antibody against the KIF11, GHSR1b or NTSR1 polypeptide, using a substance specifically binding to these epitopes, such as glutathione-Sepharose 4B.

Immunoprecipitation can be performed by following or according to, for example, the methods in the literature (Harlow and Lane, Antibodies, 511-52, Cold Spring Harbor Laboratory publications, New York (1988)).

SDS-PAGE is commonly used for analysis of immunoprecipitated proteins and the bound protein can be analyzed by the molecular weight of the protein using gels with an appropriate concentration. Since the protein bound to the KIF11, GHSR1b or NTSR1 polypeptide is difficult to detect by a common staining method, such as Coomassie staining or silver staining, the detection sensitivity for the protein can be improved by culturing cells in culture medium containing radioactive isotope, ^{35}S-methionine or ^{35}S-cystein, labeling proteins in the cells, and detecting the proteins. The target protein can be purified directly from the SDS-polyacrylamide gel and its sequence can be determined, when the molecular weight of the protein has been revealed.

As a method for screening proteins binding to any of KIF11, GHSR1b and NTSR1 polypeptides using the polypeptide, for example, West-Western blotting analysis (Skolnik et al., Cell 65: 83-90 (1991)) can be used. Specifically, a protein binding to KIF11, GHSR1b or NTSR1 polypeptide can be obtained by preparing a cDNA library from cells, tissues, organs (for example, tissues such as lung cells) or cultured cells (particularly those derived from NSCLC cells) expected to express a protein binding to the KIF11, GHSR1b or NTSR1 polypeptide using a phage vector (e.g., ZAP), expressing the protein on LB-agarose, fixing the protein expressed on a filter, reacting the purified and labeled KIF11, GHSR1b or NTSR1 polypeptide with the above filter, and detecting the plaques expressing proteins bound to the KIF11, GHSR1b or NTSR1 polypeptide according to the label. The KIF11, GHSR1b or NTSR1 polypeptide may be labeled by utilizing the binding between biotin and avidin, or by utilizing an antibody that specifically binds to the KIF11, GHSR1b or NTSR1 polypeptide, or a peptide or polypeptide (for example, GST) that is fused to the KIF11, GHSR1b or NTSR1 polypeptide. Methods using radioisotope or fluorescence and such may be also used.

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references "Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

In the two-hybrid system, KIF11, GHSR1b or NTSR1 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. A cDNA library is prepared from cells expected to express a protein binding to the KIF11, GHSR1b or NTSR1 polypeptide, such that the library, when expressed, is fused to the VP16 or GAL4 transcriptional activation region. The cDNA library is then introduced into the above yeast cells and the cDNA derived from the library is isolated from the positive clones detected (when a protein binding to the polypeptide of the invention is expressed in yeast cells, the binding of the two activates a reporter gene, making positive clones detectable). A protein encoded by the cDNA can be prepared by introducing the cDNA isolated above to *E. coli* and expressing the protein.

As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used in addition to the HIS3 gene.

A compound binding to KIF11, GHSR1b or NTSR1 polypeptide can also be screened using affinity chromatography. For example, KIF11, GHSR1b or NTSR1 polypeptide may be immobilized on a carrier of an affinity column, and a test compound, containing a protein capable of binding to KIF11, GHSR1b or NTSR1 polypeptide, is applied to the column. A test compound herein may be, for example, cell extracts, cell lysates, etc. After loading the test compound, the column is washed, and compounds bound to KIF11, GHSR1b or NTSR1 polypeptide can be prepared.

When the test compound is a protein, the amino acid sequence of the obtained protein is analyzed, an oligo DNA is synthesized based on the sequence, and cDNA libraries are screened using the oligo DNA as a probe to obtain a DNA encoding the protein.

A biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound compound in the present invention. When such a biosensor is used, the interaction between KIF11, GHSR1b or NTSR1 polypeptide and a test compound can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia). Therefore, it is possible to evaluate the binding between KIF11, GHSR1b or NTSR1 polypeptide and a test compound

using a biosensor such as BIAcore.

The methods of screening for molecules that bind when an immobilized KIF11, GHSR1b or NTSR1 polypeptide is exposed to synthetic chemical compounds, or natural substance banks or a random phage peptide display library, and the methods of screening using high-throughput based on combinatorial chemistry techniques (Wrighton et al., Science 273: 458-64 (1996); Verdine, Nature 384: 11-13 (1996); Hogan, Nature 384: 17-9 (1996)) to isolate not only proteins but chemical compounds that bind to KIF11, GHSR1b or NTSR1 protein (including agonist and antagonist) are well known to one skilled in the art.

Alternatively, the present invention provides a method of screening for a compound for treating or preventing NSCLC using KIF11, GHSR1b or NTSR1 polypeptide comprising the steps as follows:

(a) contacting a test compound with KIF11, GHSR1b or NTSR1 polypeptide;

(b) detecting the biological activity of the KIF11, GHSR1b or NTSR1 polypeptide of step (a); and

(c) selecting a compound that suppresses the biological activity of the KIF11, GHSR1b or NTSR1 polypeptide in comparison with the biological activity detected in the absence of the test compound.

Since proteins encoded by any of the genes of KIF11, GHSR1b and NTSR1 have the activity of promoting cell proliferation of NSCLC cells, a compound which inhibits this activity of one of these proteins can be screened using this activity as an index.

Any polypeptides can be used for screening so long as they comprise the biological activity of KIF11, GHSR1b or NTSR1 proteins. Such biological activity includes cell-proliferating activity and binding ability to other proteins of the proteins encoded by KIF11, GHSR1b or NTSR1 gene. For example, a human protein encoded by KIF11, GHSR1b or NTSR1 gene can be used and polypeptides functionally equivalent to these proteins can also be used. Such polypeptides may be expressed endogenously or exogenously by cells.

The compound isolated by this screening is a candidate for antagonists of the KIF11, GHSR1b or NTSR1 polypeptide. The term "antagonist" refers to molecules that inhibit the function of KIF11, GHSR1b or NTSR1 polypeptide by binding thereto. Moreover, a compound isolated by this screening is a candidate for compounds which inhibit the *in vivo* interaction of KIF11, GHSR1b or NTSR1 polypeptide with molecules (including DNAs and proteins).

When the biological activity to be detected in the present method is cell proliferation, it can be detected, for example, by preparing cells which express KIF11, GHSR1b or NTSR1 polypeptide, culturing the cells in the presence of a test compound, and determining the speed of cell proliferation, measuring the cell cycle and such, as well as by measuring the colony forming activity.

As discussed in detail above, by controlling the expression levels of KIF11, GHSR1b or NTSR1 gene, one can control the onset and progression of NSCLC. Thus, compounds that may be used in the treatment or prevention of NSCLC, can be identified through screenings that use the expression levels of one or more of KIF11, GHSR1b and NTSR1 genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

(a) contacting a test compound with a cell expressing one or more of KIF11, GHSR1b and NTSR1 genes; and

(b) selecting a compound that reduces the expression level of one or more of the genes in comparison with the expression level detected in the absence of the test compound.

Cells expressing at least one of KIF11, GHSR1b and NTSR1 genes include, for example, cell lines established from NSCLC cells; such cells can be used for the above screening of the present invention (*e.g.*, A549, NCI-H226, NCI-H522, LC319). The expression level can be estimated by methods well known to one skilled in the art. In the method of screening, a compound that reduces the expression level of at least one of the genes can be selected as candidate agents to be used for the treatment or prevention of NSCLC.

Alternatively, the screening method of the present invention may comprise the following steps:

(a) contacting a test compound with a cell into which a vector comprising the transcriptional regulatory region of one or more of the marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the marker genes are selected from the group of KIF11, GHSR1b and NTSR1;

(b) measuring the activity of said reporter gene; and

(c) selecting a compound that reduces the expression level of said reporter gene as compared to a control.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared using the transcriptional regulatory region of a

marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be isolated from a genome library based on the nucleotide sequence information of the marker gene (e.g., based the 5' upstream sequence information).

In a further embodiment of the method of screening for a compound for treating or preventing NSCLC of the present invention, the method utilizes the binding ability of KIF11 to KOC1, or GHSR1b or NTSR1 to NMU.

As described above, the present inventors revealed that KOC1 not only co-localized with KIF11 in human normal tissues, NSCLCs, and cell lines, but also directly interacted with KIF11 in NSCLC cells *in vitro*, and that the treatment of NSCLC cells with siRNAs for KIF11 reduced its expression and led to growth suppression. The results suggest that KOC1-KIF11 signaling affects growth of NSCLC cells. Thus, it is expected that the inhibition of the binding between KOC1 and KIF11 leads to the suppression of cell proliferation, and compounds inhibiting the binding serve as pharmaceuticals for treating or preventing NSCLCs. This screening method includes the steps of: (a) contacting a KIF11 polypeptide or functional equivalent thereof with KOC1 in the presence of a test compound; (b) detecting the binding between the polypeptide and KOC1; and (c) selecting the test compound that inhibits the binding between the polypeptide and KOC1.

Furthermore, as described above, the present inventors revealed GHSR1b and NTSR1 as the likely targets for the growth-promoting effect of NMU in lung tumors. The present inventors revealed that NMU-25 bound to these receptors on the cell surface, and that treatment of NSCLC cells with siRNAs for GHSR1 or NTSR1 reduced expression of the receptors and led to apoptosis. The results suggest that NMU affects growth of NSCLC cells by acting through GHSR1b and/or NTSR1 (Fig. 11). Thus, it is expected that the inhibition of binding between GHSR1b or NTSR1 and NMU leads to the suppression of cell proliferation, and compounds inhibiting the binding serve as pharmaceuticals for treating or preventing NSCLCs. This screening method includes the steps of: (a) contacting a GHSR1b or NTSR1 polypeptide or functional equivalent thereof with NMU in the presence of a test compound; (b) detecting binding between the polypeptide and NMU; and (c) selecting the test compound that inhibits binding between the polypeptide and NMU.

KOC1 and KIF11 polypeptides, or GHSR1b or NTSR1 and NMU polypeptides to be used for the screening may be a recombinant polypeptide or a protein derived from the nature, or may

also be a partial peptide thereof so long as it retains the binding ability to each other. Such partial peptides retaining the binding ability are herein referred to as "functional equivalents". The KOC1 and KIF11 polypeptides, or GHSR1b or NTSR1 and NMU polypeptides to be used in the screening can be, for example, a purified polypeptide, a soluble protein, a form bound to a carrier or a fusion protein fused with other polypeptides.

As a method of screening for compounds that inhibit binding between KOC1 and KIF11, or GHSR1b or NTSR1 and NMU, many methods well known by one skilled in the art can be used. Such a screening can be carried out as an *in vitro* assay system, for example, in a cellular system. More specifically, first, either KOC1 or KIF11, or GHSR1b or NTSR1, or NMU is bound to a support, and the other protein is added together with a test compound thereto. Next, the mixture is incubated, washed and the other protein bound to the support is detected and/or measured.

Examples of supports that may be used for binding proteins include insoluble polysaccharides, such as agarose, cellulose and dextran; and synthetic resins, such as polyacrylamide, polystyrene and silicon; preferably commercial available beads and plates (e.g., multi-well plates, biosensor chip, etc.) prepared from the above materials may be used. When using beads, they may be filled into a column. Alternatively, the use of magnetic beads of also known in the art, and enables to readily isolate proteins bound on the beads via magnetism.

The binding of a protein to a support may be conducted according to routine methods, such as chemical bonding and physical adsorption. Alternatively, a protein may be bound to a support via antibodies specifically recognizing the protein. Moreover, binding of a protein to a support can be also conducted by means of avidin and biotin.

The binding between proteins is carried out in buffer, for example, but are not limited to, phosphate buffer and Tris buffer, as long as the buffer does not inhibit binding between the proteins.

In the present invention, a biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound protein. When such a biosensor is used, the interaction between the proteins can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia). Therefore, it is possible to evaluate binding between the KOC1 and KIF11, or GHSR1b or NTSR1 and NMU using a biosensor such as BIAcore.

Alternatively, either KOC1 or KIF11, or GHSR1b or NTSR1, or NMU may be labeled, and the label of the bound protein may be used to detect or measure the bound protein.

Specifically, after pre-labeling one of the proteins, the labeled protein is contacted with the other protein in the presence of a test compound, and then bound proteins are detected or measured according to the label after washing.

Labeling substances such as radioisotope (e.g., ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{125}I, ^{131}I), enzymes (e.g., alkaline phosphatase, horseradish peroxidase, β-galactosidase, β-glucosidase), fluorescent substances (e.g., fluorescein isothiosyanete (FITC), rhodamine) and biotin/avidin, may be used for the labeling of a protein in the present method. When the protein is labeled with radioisotope, the detection or measurement can be carried out by liquid scintillation. Alternatively, proteins labeled with enzymes can be detected or measured by adding a substrate of the enzyme to detect the enzymatic change of the substrate, such as generation of color, with absorptiometer. Further, in case where a fluorescent substance is used as the label, the bound protein may be detected or measured using fluorophotometer.

Furthermore, binding of KOC1 and KIF11, or GHSR1b or NTSR1 and NMU can be also detected or measured using antibodies to the KOC1 and KIF11, or GHSR1b or NTSR1 and NMU. For example, after contacting the KOC1 polypeptide immobilized on a support with a test compound and KIF11, the mixture is incubated and washed, and detection or measurement can be conducted using an antibody against KIF11. Alternatively, KIF11 may be immobilized on a support, and an antibody against KOC1 may be used as the antibody. When the combination of GHSR1b or NTSR1 and NMU is used, GHSR1b or NTSR1 polypeptide may be immobilized on a support with a test compound and NMU, the mixture is incubated and washed, and detection or measurement can be conducted using an antibody against NMU. Alternatively, NMU may be immobilized on a support, and an antibody against GHSR1b or NTSR1 may be used as the antibody.

In case of using an antibody in the present screening, the antibody is preferably labeled with one of the labeling substances mentioned above, and detected or measured based on the labeling substance. Alternatively, the antibody against KOC1 or KIF11, or GHSR1b or NTSR1, or NMU may be used as a primary antibody to be detected with a secondary antibody that is labeled with a labeling substance. Furthermore, the antibody bound to the protein in the screening of the present invention may be detected or measured using protein G or protein A column.

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references

"Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

In the two-hybrid system, for example, KOC1 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. KIF11 polypeptide that binds to KOC1 polypeptide is fused to the VP16 or GAL4 transcriptional activation region and also expressed in the yeast cells in the existence of a test compound. Alternatively, KIF11 polypeptide may be fused to the SRF-binding region or GAL4-binding region, and KOC1 polypeptide to the VP16 or GAL4 transcriptional activation region. When the combination of GHSR1b or NTSR1 and NMU is used in the two-hybrid system, for example, GHSR1b or NTSR1 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. NMU polypeptide that binds to GHSR1b or NTSR1 polypeptide is fused to the VP16 or GAL4 transcriptional activation region and also expressed in the yeast cells in the existence of a test compound. Alternatively, NMU polypeptide may be fused to the SRF-binding region or GAL4-binding region, and GHSR1b or NTSR1 polypeptide to the VP16 or GAL4 transcriptional activation region. When the test compound does not inhibit the binding between KOC1 and KIF11, or GHSR1b or NTSR1 and NMU, the binding of the two activates a reporter gene, making positive clones detectable.

As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used besides HIS3 gene.

Moreover, when the combination of GHSR1b or NTSR1 and NMU is used in the screening method, since GHSR1b and NTSR1 are polypeptides naturally expressed on the cell surface, in a preferable embodiment of the present screening method, the polypeptides are expressed on the surface of a living cell. When the polypeptides are expressed on the surface of a living cell, the binding between the polypeptide and NMU can be detected by methods detecting the autocrine and paracrine signaling leading to stimulation of tumor cell growth (Heasley, Oncogene 20: 1563-1569 (2001)). For example, the binding between GHSR1 or NTSR1 polypeptide and NMU can be detected by:

(1) detecting the concentration of calcium or cAMP in the cell (*e.g.* FLIPR assay, Biochem. Biophys. Res. Commun. 276: 435-438, 2000; Nature 406: 70-74, 2000; J. Biol. Chem. 275:21068-21074, 2000);

(2) detecting the activation of the polypeptide;

(3) detecting the interaction between the polypeptide and G-protein (*e.g.* FLIPR assay, Biochem. Biophys. Res. Commun. 276: 435-438, 2000; Nature 406: 70-74, 2000; J. Biol. Chem.

275:21068-21074, 2000, or binding assay with ^{125}I labeled peptide);

(4) detecting the activation of phospholipase C or its down stream pathway (Oncogene 20:1563-1569, 2001);

(5) detecting the activation of kinases of the protein kinase cascade, such as Raf, MEK, ERKs, and protein kinase D (PKD) (Oncogene 20:1563-1569, 2001);

(6) detecting the activation of a member of Src/Tec/Bmx-family of tyrosine kinases (Oncogene 20:1563-1569, 2001);

(7) detecting the activation of a member of the Ras and Rho family, regulation of a member of the JNK members of MAP families, or the reorganization of the actin cytoskeleton (Oncogene 20:1563-1569, 2001);

(8) detecting the activation of any signal complex mediated by the polypeptide activation;

(9) detecting the change in subcellular localization of the polypeptide including the ligand-induced internalization/endocytosis of the polypeptide (J. Cell Sci., 113: 2963-2975, 2000; J. Histochem. Cytochem. 48:1553-1563, 2000; Endocrinology October 23, 2003. as doi: 10. 1210/en. 2003-0974)

(10) detecting the activation of any transcription factor downstream of the polypeptides or the activation of their downstream gene; and

(11) detecting cell proliferation, transformation, or any other oncogenic phenotype of the cell.

Any test compound, for example, cell extracts, cell culture supernatant, products of fermenting microorganism, extracts from marine organism, plant extracts, purified or crude proteins, peptides, non-peptide compounds, synthetic micromolecular compounds and natural compounds can be used in the screening methods of the present invention. The test compound of the present invention can be also obtained using any of the numerous approaches in combinatorial library methods known in the art, including (1) biological libraries, (2) spatially addressable parallel solid phase or solution phase libraries, (3) synthetic library methods requiring deconvolution, (4) the "one-bead one-compound" library method and (5) synthetic library methods using affinity chromatography selection. The biological library methods using affinity chromatography selection is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, Anticancer Drug Des. 12: 145 (1997)). Examples of methods for the synthesis of molecular libraries can be found in the art (DeWitt et al., Proc. Natl. Acad. Sci.

USA 90: 6909 (1993); Erb et al., Proc. Natl. Acad. Sci. USA 91: 11422 (1994); Zuckermann et al., J. Med. Chem. 37: 2678 (1994); Cho et al., Science 261: 1303 (1993); Carell et al., Angew. Chem. Int. Ed. Engl. 33: 2059 (1994); Carell et al., Angew. Chem. Int. Ed. Engl. 33: 2061 (1994); Gallop et al., J. Med. Chem. 37: 1233 (1994)). Libraries of compounds may be presented in solution (see Houghten, Bio/Techniques 13: 412 (1992)) or on beads (Lam, Nature 354: 82 (1991)), chips (Fodor, Nature 364: 555 (1993)), bacteria (US Pat. No. 5,223,409), spores (US Pat. No. 5,571,698;5,403,484, and 5,223,409), plasmids (Cull et al., Proc. Natl. Acad. Sci. USA 89: 1865 (1992)) or phage (Scott and Smith, Science 249: 386 (1990); Delvin, Science 249: 404 (1990); Cwirla et al., Proc. Natl. Acad. Sci. USA 87: 6378 (1990); Felici, J. Mol. Biol. 222: 301 (1991); US Pat. Application 2002103360). The test compound exposed to a cell or protein according to the screening methods of the present invention may be a single compound or a combination of compounds. When a combination of compounds are used in the screening methods of the invention, the compounds may be contacted sequentially or simultaneously.

A compound isolated by the screening methods of the present invention is a candidate for drugs which inhibit the activity of KIF11, GHSR1b or NTSR1 polypeptide, for treating or preventing diseases attributed to, for example, cell proliferative diseases, such as NSCLC. A compound in which a part of the structure of the compound obtained by the present screening methods of the present invention is converted by addition, deletion and/or replacement, is included in the compounds obtained by the screening methods of the present invention. A compound effective in suppressing the expression of over-expressed genes, i.e., KIF11, GHSR1b or NTSR1 gene, is deemed to have a clinical benefit and can be further tested for its ability to prevent cancer cell growth in animal models or test subjects.

Selecting a therapeutic agent for treating and/or preventing NSCLC that is appropriate for a particular individual

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. A compound that is metabolized in a subject to act as an anti-NSCLC agent can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of a cancerous state to a gene expression pattern characteristic of a non-cancerous state. Accordingly, the differentially expressed KIF11, GHSR1b and NTSR1 genes disclosed herein allow for selection of a putative therapeutic or prophylactic inhibitor of NSCLC specifically adequate for a subject by testing candidate compounds in a test cell (or test cell population) derived from the selected subject.

To identify an anti-NSCLC agent, that is appropriate for a specific subject, a test cell or test cell population derived from the subject is exposed to a therapeutic agent and the expression of one or more of the KIF11, GHSR1b and NTSR1 genes is determined.

The test cell is or the test cell population contains an NSCLC cell expressing an NSCLC-associated gene. Preferably, the test cell is or the test cell population contains a lung cell. For example, the test cell or test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test cell or cell population is measured and compared to one or more reference profiles, *e.g.*, an NSCLC reference expression profile or an non-NSCLC reference expression profile.

A decrease in the expression of one or more of KIF11, GHSR1b and NTSR1 in a test cell or test cell population relative to a reference cell population containing NSCLC is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agent is an immunomodulatory agent.

Methods for treating or preventing NSCLC

The present invention provides a method for treating, alleviating or preventing NSCLC in a subject. Therapeutic compounds are administered prophylactically or therapeutically to subjects suffering from or at risk of (or susceptible to) developing NSCLC. Such subjects are identified using standard clinical methods or by detecting an aberrant level of expression or activity of KIF11, GHSR1b or NTSR1 gene or polypeptide. Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or alternatively delayed in its progression.

The method includes decreasing the expression or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("over-expressed gene"; KIF11, GHSR1b or NTSR1 gene) in an NSCLC cell relative to normal cells of the same tissue type from which the NSCLC cells are derived. The expression may be inhibited by any method known in the art. For example, a subject may be treated with an effective amount of a compound that decreases the amount of one or more of the KIF11, GHSR1b or NTSR1 gene in the subject. Administration of the compound can be systemic or local. Such therapeutic compounds include compounds that decrease the expression level of such gene that endogenously exists in the NSCLC cells (*i.e.*, compounds that down-regulate the expression of the over-expressed gene(s), KIF11, GHSR1b and/or NTSR1 genes). The administration of

such therapeutic compounds counter the effects of aberrantly-over expressed gene(s) in the subjects NSCLC cells and are expected to improve the clinical condition of the subject. Such compounds can be obtained by the screening method of the present invention described above.

The compounds that modulate the activity of a protein encoded by KIF11, GHSR1b or NTSR1 gene that can be used for treating or preventing NSCLC of the present invention include besides proteins, naturally-occurring cognate ligand of these proteins, peptides, peptidomimetics and other small molecules.

Alternatively, the expression of these over-expressed gene(s) (KIF11, GHSR1b and/or NTSR1) can be inhibited by administering to the subject a nucleic acid that inhibits or antagonizes the expression of the over-expressed gene(s). Antisense oligonucleotides, siRNAs or ribozymes which disrupt the expression of the over-expressed gene(s) can be used for inhibiting the expression of the over-expressed gene(s).

As noted above, antisense-oligonucleotides corresponding to any of the nucleotide sequence of KIF11, GHSR1b or NTSR1 gene can be used to reduce the expression level of the gene. Antisense-oligonucleotides corresponding to KIF11, GHSR1b and NTSR1 genes that are up-regulated in NSCLC are useful for the treatment or prevention of NSCLC. Specifically, the antisense-oligonucleotides against the genes may act by binding to any of the corresponding polypeptides encoded by these genes, or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the mRNAs, and/or inhibiting the expression of proteins encoded by the KIF11, GHSR1b and NTSR1 nucleotides, and finally inhibiting the function of the proteins. The term "antisense-oligonucleotides" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense-oligonucleotides can specifically hybridize to the target sequence. For example, the antisense-oligonucleotides of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably 95% or higher over a span of at least 15 continuous nucleotides to any of the nucleotide sequence of KIF11, GHSR1b or NTSR1 gene. Algorithms known in the art can be used to determine the homology. Furthermore, derivatives or modified products of the antisense-oligonucleotides can also be used as antisense-oligonucleotides in the present invention. Examples of such modified products include lower alkyl phosphonate modifications such as methyl-phosphonate-type or ethyl-phosphonate-type, phosphorothioate modifications and phosphoroamidate modifications.

The antisense-oligonucleotides and derivatives thereof act on cells producing the proteins

encoded by KIF11, GHSR1b or NTSR1 gene by binding to the DNA or mRNA encoding the protein, inhibiting transcription or translation thereof, promoting the degradation of the mRNAs and inhibiting the expression of the protein, thereby resulting in the inhibition of the protein function.

An antisense-oligonucleotides and derivatives thereof can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

The antisense-oligonucleotides of the invention inhibit the expression of at least one protein encoded by any one of KIF11, GHSR1b and NTSR1 genes, and thus are useful for suppressing the biological activity of the protein.

The polynucleotides that inhibit one or more gene products of over-expressed genes also include small interfering RNAs (siRNA) comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid of the nucleotide sequence encoding an over-expressed protein encoded by KIF11, GHSR1b or NTSR1 gene. The term "siRNA" refers to a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell can be used in the treatment or prevention of the present invention, including those in which DNA is a template from which RNA is transcribed. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, *e.g.*, a hairpin.

The method is used to suppress gene expression of a cell with up-regulated expression of KIF11, GHSR1b or NTSR1 gene. Binding of the siRNA to KIF11, GHSR1b or NTSR1 gene transcript in the target cell results in a reduction of KIF11, GHSR1b or NTSR1 protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally occurring transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 or 25 nucleotides in length. Preferable siRNA of the present invention include the polynucleotides having the nucleotide sequence of SEQ ID NO: 32, 33, 34, 37, 39, or 40 as the target sequence, which all proved to be effective for suppressing cell viability of NSCLC cell lines. Specifically, a preferable siRNA used in the present invention has the general formula:

5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a target sequence of KIF11, GHSR1b or NTSR1; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A]. Herein, the phrase a "target sequence

of KIF11, GHSR1b or NTSR1 gene" refers to a sequence that, when introduced into NSCLC cell lines, is effective for suppressing cell viability. Preferred target sequence of KIF11, GHSR1b or NTSR1 gene includes nucleotide sequences comprising: SEQ ID NOs: 32, 33, 34, 37, 39, and 40. The complementary sequence [A'] and [A] hybridize to each other to form a double strand, and the whole siRNA molecule with the general formula 5'-[A]-[B]-[A']-3' forms a hairpin loop structure. As used herein, the term "complementary" refers to a Watson-Crick or Hoogsteen base pairing between nucleotide units of a polynucleotide, and hybridization or binding of nucleotide units indicates physical or chemical interaction between the units under appropriate conditions to form a stable duplex (double-stranded configuration) containing few or no mismatches. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 base pairs. Particularly preferred duplexes are fully complementary and contain no mismatch. The siRNA against the mRNA of KIF11, GHSR1b or NTSR1 gene to be used in the present invention contains a target sequence shorter than the whole mRNA of KIF11, GHSR1b or NTSR1 gene, and has a sequence of 500, 200, or 75 nucleotides as the whole length. Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors. The isolated nucleic acids of the present invention are useful for siRNA against KIF11, GHSR1b or NTSR1 or DNA encoding the siRNA. When the nucleic acids are used for siRNA or coding DNA thereof, the sense strand is preferably longer than 19 nucleotides, and more preferably longer than 21 nucleotides.

Furthermore, the nucleotide sequence of siRNAs may be designed using a siRNA design computer program available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The nucleotide sequences for the siRNA are selected by the computer program based on the following protocol:

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend not to design siRNA against the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites, and thus the complex of endonuclease and siRNAs that were designed against these regions may interfere with the binding of UTR-binding proteins and/or translation initiation complexes.

2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences.

The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/

3. Select qualifying target sequences for synthesis. On the website of Ambion, several preferable target sequences can be selected along the length of the gene for evaluation.

The siRNAs inhibit the expression of over-expressed KIF11, GHSR1b or NTSR1 protein and is thereby useful for suppressing the biological activity of the protein. Therefore, a composition comprising the siRNA is useful in treating or preventing non-small cell lung cancer.

The nucleic acids that inhibit one or more gene products of over-expressed genes KIF11, GHSR1b and NTSR1 also include ribozymes against the gene(s).

The ribozymes inhibit the expression of over-expressed KIF11, GHSR1b or NTSR1 protein and is thereby useful for suppressing the biological activity of the protein. Therefore, a composition comprising the ribozyme is useful in treating or preventing NSCLC.

Generally, ribozymes are classified into large ribozymes and small ribozymes. A large ribozyme is known as an enzyme that cleaves the phosphate ester bond of nucleic acids. After the reaction with the large ribozyme, the reacted site consists of a 5'-phosphate and 3'-hydroxyl group. The large ribozyme is further classified into (1) group I intron RNA catalyzing transesterification at the 5'-splice site by guanosine; (2) group II intron RNA catalyzing self-splicing through a two step reaction via lariat structure; and (3) RNA component of the ribonuclease P that cleaves the tRNA precursor at the 5' site through hydrolysis. On the other hand, small ribozymes have a smaller size (about 40 bp) compared to the large ribozymes and cleave RNAs to generate a 5'-hydroxyl group and a 2'-3' cyclic phosphate. Hammerhead type ribozymes (Koizumi et al., FEBS Lett. 228: 225 (1988)) and hairpin type ribozymes (Buzayan, Nature 323: 349 (1986); Kikuchi and Sasaki, Nucleic Acids Res. 19: 6751 (1992)) are included in the small ribozymes. Methods for designing and constructing ribozymes are known in the art (see Koizumi et al., FEBS Lett. 228: 225 (1988); Koizumi et al., Nucleic Acids Res. 17: 7059 (1989); Kikuchi and Sasaki, Nucleic Acids Res. 19: 6751 (1992)) and ribozymes inhibiting the expression of an over-expressed NSC protein can be constructed based on the sequence information of the nucleotide sequence encoding KIF11, GHSR1b or NTSR1 protein according to conventional methods for producing ribozymes.

The ribozymes inhibit the expression of over-expressed KIF11, GHSR1b or NTSR1 protein and is thereby useful for suppressing the biological activity of the protein. Therefore, a composition comprising the ribozyme is useful in treating or preventing NSCLC.

Alternatively, the function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by any of the up-regulated genes KIF11, GHSR1b or NTSR1, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure that interacts (binds) specifically with a molecule comprising the antigen used for synthesizing the antibody (*i.e.*, the up-regulated gene product) or with an antigen closely related to it. An antibody that binds to the over-expressed KIF11, GHSR1b or NTSR1 nucleotide may be in any form, such as monoclonal or polyclonal antibodies, and includes antiserum obtained by immunizing an animal such as a rabbit with the polypeptide, all classes of polyclonal and monoclonal antibodies, human antibodies and humanized antibodies produced by genetic recombination. Furthermore, the antibody used in the method of treating or preventing NSCLC of the present invention may be a fragment of an antibody or a modified antibody, so long as it binds to one or more of the proteins encoded by the marker genes (KIF11, GHSR1b or NTSR1 gene). The antibodies and antibody fragments used in the present method of treating or preventing NSCLC may be modified, and include chemically modified and chimeric antibodes. Such antibodies and antibody fragments can be obtained according to the above-mentioned methods, *supra*.

When the obtained antibody is to be administered to the human body (antibody treatment), a human antibody or a humanized antibody is preferable for reducing immunogenicity.

For example, transgenic animals having a repertory of human antibody genes may be immunized with an antigen such as KIF11, GHSR1b or NTSR1 polypeptide, cells expressing the polypeptide, or their lysates. Antibody producing cells are then collected from the animals and fused with myeloma cells to obtain hybridoma, from which human antibodies against the polypeptide can be prepared (see WO92-03918, WO93-2227, WO94-02602, WO94-25585, WO96-33735, and WO96-34096).

Alternatively, an immune cell, such as an immunized lymphocyte, producing antibodies may be immortalized by an oncogene and used for preparing monoclonal antibodies.

The present invention provides a method for treating or preventing NSCLC, using an antibody against an over-expressed KIF11, GHSR1b or NTSR1 polypeptide. According to the method, a pharmaceutically effective amount of an antibody against KIF11, GHSR1b or

NTSR1 polypeptide is administered. An antibody against an over-expressed KIF11, GHSR1b or NTSR1 polypeptide is administered at a dosage sufficient to reduce the activity of KIF11, GHSR1b or NTSR1 protein. Alternatively, an antibody binding to a cell surface marker specific for tumor cells can be used as a tool for drug delivery. Thus, for example, an antibody against an over-expressed KIF11, GHSR1b or NTSR1 polypeptide conjugated with a cytotoxic agent may be administered at a dosage sufficient to injure tumor cells.

The present invention also relates to a method of treating or preventing NSCLC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of KIF11, GHSR1b and NTSR1 genes or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. Administration of the polypeptide induces an anti-tumor immunity in a subject. Thus, the present invention further provides a method for inducing anti tumor immunity. The polypeptide or the immunologically active fragments thereof are useful as vaccines against NSCLC. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell receptor (TCR) or presented on an antigen presenting cell (APC), such as macrophage, dendritic cell (DC) or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, the phrase "vaccine against NSCLC" refers to a substance that has the function to induce anti-tumor immunity or immunity to suppress NSCLC upon inoculation into animals. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing *in vivo* or *in vitro* the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells).

Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils and NK cells. Since CD4+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using ^{3}H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported to be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL is shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against NSCLC. Furthermore, APC that acquired the ability to induce CTL against NSCLC by contacting with the polypeptides are useful as vaccines against NSCLC. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against NSCLC. Such therapeutic methods for NSCLC using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and

when growth, proliferation or metastasis of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of NSCLC. Therapy against or prevention of the onset of NSCLC includes any of the steps, such as inhibition of the growth of NSCLC cells, involution of NSCLC cells and suppression of occurrence of NSCLC cells. Decrease in mortality of individuals having NSCLC, decrease of marker genes (in addition to KIF11, GHSR1b and/or NTSR1 genes) in the blood, alleviation of detectable symptoms accompanying NSCLC and such are also included in the therapy or prevention of NSCLC. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against NSCLC is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test or ANOVA may be used for statistical analysis.

The above-mentioned protein having immunological activity, or a polynucleotide or vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, NSCLC can be treated or prevented, for example, by the *ex vivo* method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide *ex vivo*, and following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the polypeptide into PBMCs *ex vivo*. APC or CTL induced *in vitro* can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against

similar types of diseases in other individuals.

Moreover, the present invention provides a method for treating or preventing NSCLC in a subject, wherein a compound obtained according to any of the above-described screening methods is administered to the subject. Any compound that are obtained according to any of the screening methods of the present invention can be administered to the subject so long as it decreases the expression or function, or both, of one or more gene products of KIF11, GHSR1b and NTSR1 genes.

siRNA and vectors encoding them

Transfection of vectors expressing siRNA for KIF11, GHSR1b or NTSR1 leads to growth inhibition of NSCLC cells. Thus; it is another aspect of the present invention to provide a double-stranded molecule comprising a sense-strand and antisense-strand which molecule functions as an siRNA for KIF11, GHSR1b or NTSR1, and a vector encoding the double-stranded molecule.

The double-stranded molecule of the present invention comprises a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a KIF11, GHSR1b, or NTSR1 target sequence, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing a KIF11, GHSR1b, or NTSR1 gene, inhibits expression of said gene.

The double-stranded molecule of the present invention may be a polynucleotide derived from its original environment (i.e., when it is a naturally occurring molecule, the natural environment), physically or chemically altered from its natural state, or chemically synthesized. According to the present invention, such double-stranded molecules include those composed of DNA, RNA, and derivatives thereof. A DNA is suitably composed of bases such as A, T, C and G, and T is replaced by U in an RNA.

As described above, the term "complementary" refers to a Watson-Crick or Hoogsteen base pairing between nucleotide units of a polynucleotide, and hybridization or binding of nucleotide units indicates physical or chemical interaction between the units under appropriate conditions to form a stable duplex (double-stranded configuration) containing few or no mismatches. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 base

pairs. Particularly preferred duplexes are fully complementary and contain no mismatch.

The double-stranded molecule of the present invention contains a ribonucleotide sequence corresponding to a KIF11, GHSR1b, or NTSR1 target sequence shorter than the whole mRNA of KIF11, GHSR1b or NTSR1 gene. Herein, the phrase a "target sequence of KIF11, GHSR1b or NTSR1 gene" refers to a sequence that, when introduced into NSCLC cell lines, is effective for suppressing cell viability. Specifically, the target sequence comprises at least about 10, or suitably about 19 to about 25 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3 and 5. That is, the sense strand of the present double-stranded molecule consists of at least about 10 nucleotides, suitably is longer than 19 nucleotides, and more preferably longer than 21 nucleotides. Preferred target sequences include the sequences of SEQ ID NOs: 32, 33, 34, 37, 39, and 40. The present double-stranded molecule including the sense strand and the antisense strand is an oligonucleotide shorter than about 100, preferably 75, more preferably 50 and most preferably 25 nucleotides in length. A suitable double-stranded molecule of the present invention is an oligonucleotide of a length of about 19 to about 25 nucleotides. Furthermore, in order to enhance the inhibition activity of the siRNA, nucleotide "u" can be added to 3'end of the antisense strand of the target sequence. The number of "u"s to be added is at least 2, generally 2 to 10, preferably 2 to 5. The added "u"s form single strand at the 3'end of the antisense strand of the siRNA.

Furthermore, the double-stranded molecule of the present invention may be a single ribonucleotide transcript comprising the sense strand and the antisense strand linked via a single-stranded ribonucleotide sequence. Namely, the present double-stranded molecule may have the general formula:

5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a target sequence of KIF11, GHSR1b or NTSR1;

[B] is a ribonucleotide sequence (loop sequence) consisting of 3 to 23 nucleotides; and

[A'] is a ribonucleotide sequence complementary to [A]. The complementary sequence [A'] and [A] hybridize to each other to form a double strand, and the whole siRNA molecule with the general formula 5'-[A]-[B]-[A']-3' forms a hairpin loop structure.

The region [A] hybridizes to [A'], and then a loop consisting of region [B] is formed. The loop sequence can be selected from those describe in

http://www.ambion.com/techlib/tb/tb_506.html, or those described in Jacque, J.-M., Triques, K., and Stevenson, M. "Modulation of HIV-1 replication by RNA interference." Nature 418: 435-438 (2002). Additional examples of the loop sequence that can be included in the present double-stranded molecules include:

CCC, CCACC or CCACACC: Jacque, J. M., Triques, K., and Stevenson, M. "Modulation of HIV-1 replication by RNA interference." Nature, Vol. 418: 435-438 (2002);

UUCG: Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A., Salvaterra, P., and Rossi, J. "Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells." Nature Biotechnology 20:500-505 (2002); Fruscoloni, P., Zamboni, M., and Tocchini-Valentini, G. P. "Exonucleolytic degradation of double-stranded RNA by an activity in Xenopus laevis germinal vesicles." Proc. Natl. Acad. Sci. USA 100(4): 1639-1644 (2003); and

UUCAAGAGA: Dykxhoorn, D. M., Novina, C. D., and Sharp, P. A. "Killing the messenger: Short RNAs that silence gene expression." Nature Reviews Molecular Cell Biology 4: 457-467 (2002).

Preferable siRNAs having hairpin loop structure of the present invention are shown below. In the following structure, the loop sequence can be selected from the group consisting of: CCC, UUCG, CCACC, CCACACC, and UUCAAGAGA. Among these sequences, the most preferable loop sequence is UUCAAGAGA (corresponding to "ttcaagaga" in a DNA):

guuaguguacgaacuggag-[B]-cuccaguucguacacuaac (for the target sequence of SEQ ID NO:32);

gugucucugu uggagaucu-[B]-agaucuccaacagagacac (for the target sequence of SEQ ID NO:33);

gaaggcaguu gaccaacac-[B]-guguuggucaacugccuuc (for the target sequence of SEQ ID NO:34);

ccucuaccug uccagcaug-[B]-caugcuggacagguagagg (for the target sequence of SEQ ID NO:37);

guucaucagc gccaucugg-[B]-ccagauggcgcugaugaac (for the target sequence of SEQ ID NO:39); and

ggucgucaua caggucaac-[B]-guugaccuguaugacgacc (for the target sequence of SEQ ID NO:40).

The present invention further provides a vector encoding the double-stranded molecule of the present invention. The vector encodes a transcript having a secondary structure and which comprises the sense strand and the antisense strand, and suitably comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand. The vector preferably comprises a regulatory sequence adjacent to the region encoding the present double-stranded molecule that directs the expression of the molecule in an adequate cell. For example, the double-stranded molecules of the present invention are intracellularly transcribed by cloning their coding sequence into a vector containing, e.g., a RNA pol III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter.

Alternatively, the present vectors are produced, for example, by cloning the target sequence into an expression vector so the objective sequence is operatively-linked to a regulatory sequence of the vector in a manner to allow expression thereof (transcription of the DNA molecule) (Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A.,Salvaterra, P., and Rossi, J. "Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells." Nature Biotechnology 20: 500-505 (2002)). For example, the transcription of an RNA molecule having an antisense sequence to the target sequence is driven by a first promoter (e.g., a promoter sequence linked to the 3'-end of the cloned DNA) and that having the sense strand to the target sequence by a second promoter (e.g., a promoter sequence linked to the 5'-end of the cloned DNA). The expressed sense and antisense strands hybridize to each other *in vivo* to generate a siRNA construct to silence a gene that comprises the target sequence. Furthermore, two constructs (vectors) may be utilized to respectively produce the sense and anti-sense strands of a siRNA construct.

For introducing the vectors into a cell, transfection-enhancing agent can be used. FuGENE (Rochediagnostices), Lipofectamin 2000 (Invitrogen), Oligofectamin (Invitrogen), and Nucleofactor (Wako pure Chemical) are useful as the transfection-enhancing agent.

Pharmaceutical compositions for treating or preventing NSCLC

The present invention provides compositions for treating or preventing NSCLC comprising a compound selected by the present method of screening for a compound that alters the expression or activity of an NSCLC-associated gene.

When administering a compound isolated by the screening method of the present invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pig, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons or chimpanzees for treating a cell proliferative

disease (e.g., non-small cell lung cancer), the isolated compound can be directly administered or can be formulated into a dosage form using conventional pharmaceutical preparation methods. Such pharmaceutical formulations of the present compositions include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. The formulations are optionally packaged in discrete dosage units.

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Formulations also include powders, granules, solutions, suspensions or emulsions. The active ingredient is optionally administered as a bolus electuary or paste.. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made via molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle prior to use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils) or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient *in vivo*. A package of tablets may contain one tablet to be taken on each of the month. The formulation or dose of medicament in these preparations makes a suitable dosage within the indicated range acquirable.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example, buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin, glycerin, sucrose or acacia. For intra-nasal administration of an active ingredient, a liquid spray or dispersible powder or in the form of drops may be used. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compositions are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichiorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compositions may take the form of a dry powder composition, for example, a powder mix of an active ingredient and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

When desired, the above-described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, of the active ingredient or an appropriate fraction thereof.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The present invention further provides a composition for treating or preventing NSCLC comprising active ingredient that inhibits the expression of any one of the gene selected from the group of KIF11, GHSR1b and NTSR1 genes. Such active ingredient can be an antisense-oligonucleotide, siRNA or ribozyme against the gene, or derivatives, such as expression vector, of the antisense-oligonucleotide, siRNA or ribozyme. The active ingredient may be made into an external preparation, such as liniment or a poultice, by mixing with a suitable base material which is inactive against the derivatives.

Also, as needed, the active ingredient can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, preservatives, pain-killers and such. These can be prepared according to conventional methods for preparing nucleic acid containing pharmaceuticals.

Preferably, the antisense-oligonucleotide derivative, siRNA derivative or ribozyme derivative is given to the patient by direct application to the ailing site or by injection into a blood vessel so that it will reach the site of ailment. A mounting medium can also be used in the composition to increase durability and membrane-permiability. Examples of mounting mediums include liposome, poly-L-lysine, lipid, cholesterol, lipofectin and derivatives thereof.

The dosage of such compositions can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

Another embodiment of the present invention is a composition for treating or preventing NSCLC comprising an antibody against a polypeptide encoded by any one of the genes selected

from the group of KIF11, GHSR1b and NTSR1 genes or fragments of the antibody that bind to the polypeptide.

Although there are some differences according to the symptoms, the dose of an antibody or fragments thereof for treating or preventing NSCLC is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the condition of the patient, symptoms of the disease and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kg of body-weight.

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. Any patents, patent applications and publications sited herein are incorporated by reference.

BEST MODE FOR CARRING OUT THE INVENTION

Materials and methods

(1) Patients and tissue samples

Primary NSCLC samples, of which 22 were classified as adenocarcinomas (ADCs), 14 as squamous-cell carcinomas (SCCs), and one as adenosquamous carcinoma, had been obtained earlier with informed consent from 37 patients (Kikuchi, T., Daigo, Y., Katagiri, T., Tsunoda, T., Okada, K., Kakiuchi, S., Zembutsu, H., Furukawa, Y., Kawamura, M., Kobayashi, K., Imai, K., Nakamura, Y. "Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs", Oncogene 22, 2192-2205 (2003)). Fifteen additional primary NSCLCs, including

seven ADCs and eight SCCs, were obtained along with adjacent normal lung tissue samples from patients undergoing surgery at our institutes.

(2) Cell lines

Twenty-one human NSCLC cancer cell lines were grown in monolayers in appropriate medium supplemented with 5 or 10% fetal bovine serum (see Table 1).

Table 1

Cell line name		Medium
adenocarcinoma (ADC)		
	A549	RPMI-1640 (10% FBS)
	NCI-H23	RPMI-1640 (10% FBS)
	NCI-H522	RPMI-1640 (10% FBS)
	LC174	RPMI-1640 (10% FBS)
	LC176	RPMI-1640 (10% FBS)
	LC319	RPMI-1640 (10% FBS)
	PC-3	DMEM (10% FBS)
	PC-9	DMEM (10% FBS)
	PC14	RPMI-1640 (10% FBS)
	PC14-PE6	RPMI-1640 (10% FBS)
	NCI-H1373	RPMI-1640 (10% FBS)
	NCI-H1435	F12+DMEM (5%FBS)+EGF(+)
	NCI-H1793	F12+DMEM (5%FBS)+Glu
	SK-LU-1	DMEM (10% FBS)
BAC	NCI-H358	RPMI-1640 (10% FBS)
BAC	NCI-H1650	RPMI-1640 (10% FBS)
BAC	SW1573	Leibovitz's L-15 (10% FBS)
squamous cell carcinoma (SCC)		
	RERF-LC-AI	DMEM (10% FBS)
	SW-900	Leibovitz's L-15 (10% FBS)
	SK-MES-1	DMEM (10% FBS)
adenosquamous	NCI-H596	RPMI-1640 (10% FBS)

(3) Semiquantitative RT-PCR analysis

Total RNA was extracted from cultured cells and clinical tissues using Trizol reagent (Life Technologies, Inc.) according to the manufacturer's protocol. Extracted RNAs and normal human tissue poly(A) RNAs were treated with DNase I (Nippon Gene) and reverse-transcribed

using oligo(dT) primer and SuperScript II reverse transcriptase (Invitrogen). Semiquantitative RT-PCR experiments were carried out with the following synthesized gene-specific primers or with beta-actin (ACTB)-specific primers as an internal control:

KOC1, 5'-TAAATGGCTTCAGGAGACTTCAG-3' (SEQ.ID.NO.7) and
5'-GGTTTTAAATGCAGCTCCTATGTG-3' (SEQ.ID.NO.8);
KIF11, 5'-CTGAACAGTGGGTATCTTCCTTA-3' (SEQ.ID.NO.9) and
5'-GATGGCTCTTGACTTAGAGGTTC-3' (SEQ.ID.NO.10);
NMU, 5'-TGAAGAGATTCAGAGTGGACGA-3' (SEQ.ID.NO.11) and
5'-ACTGAGAACATTGACAACACAGG-3' (SEQ.ID.NO.12);
NMU1R, 5'-AAGAGGGACAGGGACAAGTAGT-3' (SEQ.ID.NO.13) and
5'-ATGCCACTGTTACTGCTTCAG-3' (SEQ.ID.NO.14);
NMU2R, 5'- GGCTCTTACAACTCATGTACCCA-3' (SEQ.ID.NO.15) and
5'-TGATACAGAGACATGAAGTGAGCA-3' (SEQ.ID.NO.16);
GHSR1a, 5'-TGGTGTTTGCCTTCATCCT-3' (SEQ.ID.NO.17) and
5'-GAATCCCAGAAGTCTGAACA-3' (SEQ.ID.NO.18);
GHSR1b, 5'-ACGGTCCTCTACAGTCTCA-3' (SEQ.ID.NO.19) and
5'-CACAGGGAGAGGATAGGA-3' (SEQ.ID.NO.20);
NTSR1, 5'-AGTGGGCTCAGAGTCTAGCAAAT-3' (SEQ.ID.NO.21) and
5'-TATTGAGAGATACACGGGGTTTG-3' (SEQ.ID.NO.22);
GHRL, 5'-TGAGCCCTGAACACCAGAGAG-3' (SEQ.ID.NO.23) and
5'-AAAGCCAGATGAGCGCTTCTA-3' (SEQ.ID.NO.24);
NTS, 5'-TCTTCAGCATGATGTGTTGTGT-3' (SEQ.ID.NO.25) and
5'-TGAGAGATTCATGAGGAAGTCTTG-3' (SEQ.ID.NO.26);
ACTB, 5'-GAGGTGATAGCATTGCTTTCG-3' (SEQ.ID.NO.27) and
5'-CAAGTCAGTGTACAGGTAAGC-3' (SEQ.ID.NO.28).

PCR reactions were optimized for the number of cycles to ensure product intensity within the logarithmic phase of amplification.

(4) Northern-blot analysis

Human multiple-tissue blots (BD Biosciences Clontech) were hybridized with ^{32}P-labeled PCR products of KOC1, KIF11 and GHSR1. cDNA probes of KOC1, KIF11 and NMU were prepared by RT-PCR using primers similarly as above. Prehybridization, hybridization, and washing were performed according to the supplier's recommendations. The blots were autoradiographed with intensifying BAS screens (BIO-RAD) at room temperature (RT) for 30 to 168 hours.

(5) RNA interference assay

To prepare plasmid vector expressing short interfering RNA (siRNA), we amplified the genomic fragment of H1RNA gene containing its promoter region by PCR using a set of primers, 5'-TGGTAGCCAAGTGCAGGTTATA-3' (SEQ ID No: 47), and

5'- CCAAAGGGTTTCTGCAGTTTCA-3' (SEQ ID No: 48) and human placental DNA as a template. The product was purified and cloned into pCR2.0 plasmid vector using a TA cloning kit according to the supplier's protocol (Invitrogen). The *Bam*HI and *Xho*I fragment containing *H1RNA* was into pcDNA3.1(+) between nucleotides 56 and 1257, and the fragment was amplified by PCR using

5'-TGCGGATCCAGAGCAGATTGTACTGAGAGT-3' (SEQ ID No: 49) and

5'- CTCTATCTCGAGTGAGGCGGAAAGAACCA-3' (SEQ ID No: 50),

The ligated DNA became the template for PCR amplification with primers,

5'- TTTAAGCTTGAAGACCATTTTTGGAAAAAAAAAAAAAAAAAAAAAC-3' (SEQ ID No: 51) and

5'-TTTAAGCTTGAAGACATGGGAAAGAGTGGTCTCA-3' (SEQ ID No: 52).

The product was digested with *Hin*dIII, and subsequently self-ligated to produce psiH 1BX3.0 vector plasmid having a nucleotide sequence shown in SEQ ID NO: 53.

The DNA flagment encoding siRNA was inserted into the GAP at nucleotide 489-492 as indicated (-) in the following plasmid sequence (SEQ ID NO: 53).

GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGGATCCA
CTAGTAACGGCCGCCAGTGTGCTGGAATTCGGCTTGGTAGCCAAGTGCAGGTTATAGGGAGC
TGAAGGGAAGGGGGTCACAGTAGGTGGCATCGTTCCTTTCTGACTGCCCGCCCCCCGCATGC
CGTCCCGCGATATTGAGCTCCGAACCTCTCGCCCTGCCGCCGCCGGTGCTCCGTCGCCGCCG
CGCCGCCATGGAATTCGAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGT
GTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC
AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGT
GAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTCTTTCCC—TTTTTGG
GAAAAAAAAAAAAAAAAAAAAAAACGAAACCGGGCCGGGCGCGGTGGTTCACGCCTATAAT
CCCAGCACTTTGGGAGGCCGAGGCGGGCGGATCACAAGGTCAGGAGGTCGAGACCATCCA
GGCTAACACGGTGAAACCCCCCCCCATCTCTACTAAAAAAAAAAAAATACAAAAAATTAGCCA
TTAGCCGGGCGTGGTGGCGGGCGCCTATAATCCCAGCTACTTGGGAGGCTGAAGCAGAATG
GCGTGAACCCGGGAGGCGGACGTTGCAGTGAGCCGAGATCGCGCCGACTGCATTCCAGCCT
GGGCGACAGAGCGAGTCTCAAAAAAAAAAACCGAGTGGAATGTGAAAAGCTCCGTGAAACT
GCAGAAACCCAAGCCGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGTGAGGCG
GAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCG
CGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGC
TCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAAT
CGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGA
TTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTT
GGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCT
CGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCT
GATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAA

GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCA
GGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTA
GTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCG
CCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTG
CCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGGAAAAAG
CTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGC
ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGG
CTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGC
AGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGA
CGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGAC
GTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCC
TGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGC
ATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCA
CGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCT
CGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTC
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTC
ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGA
TATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCG
CTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCT
GGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACC
GCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCT
CCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAA
TGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTC
TAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGC
TAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTC
CACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTA
ACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGC
TGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCT
TCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTC
AAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCA
AAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGC
TCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC
AGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGA
CCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATA
GCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCAC
GAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCC
GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG
TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAAC
AGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT
GATCCGGCAAACAAACCACCGCTGGTAGCGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGC
AGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAA
CGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCT
TTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGT
TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTG
CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCT
GCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGC
CGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATT
GTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATT
GCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAA
CGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCC
TCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCA
TAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA

GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAA
TACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAA
AACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAAC
TGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAA
TGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAG
AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTC

Using 30μl of Lipofectamine 2000 (Invitrogen), 10μg of siRNA-expression vector were transfected into NSCLC cell lines, A549 and LC319, both endogenously over-expressing KOC1, KIF11, NMU, GHSR1b, and NTSR1. More than 90% of the transfected cells expressed the synthetic siRNAs, and endogenous expression of target genes (KIF11, GHSR, or NTSR1) in these cells was effectively suppressed. The transfected cells were cultured for five days in the presence of appropriate concentrations of geneticin (G418), and then, cell numbers and viability were measured by Giemsa staining and triplicate MTT assays. The target sequences of the synthetic oligonucleotides for RNAi were as follows: control 1 (EGFP: enhanced green fluorescent protein (GFP) gene, a mutant of Aequorea victoria GFP), 5'-GAAGCAGCACGACTTCTTC-3' (SEQ.ID.NO.29); control 2 (Luciferase: Photinus pyralis luciferase gene), 5'-CGTACGCGGAATACTTCGA-3' (SEQ.ID.NO.30); control 3 (Scramble: chloroplast Euglena gracilis gene coding for 5S and 16S rRNAs), 5'-GCGCGCTTTGTAGGATTCG-3' (SEQ.ID.NO.31);
siRNA-KIF11-1 (#1), 5'-GTTAGTGTACGAACTGGAG-3' (SEQ.ID.NO.32);
siRNA-KIF11-2 (#2), 5'-GTGTCTCTGTTGGAGATCT-3' (SEQ.ID.NO.33);
siRNA-KIF11-3 (#3), 5'-GAAGGCAGTTGACCAACAC-3' (SEQ.ID.NO.34);
siRNA-KIF11-4 (#4), 5'-GAGACTGAACAGAGATGTG-3' (SEQ.ID.NO.35);
siRNA-KIF11-5 (#5), 5'-GCCAATGTTGTGAGGCTTC-3' (SEQ.ID.NO.36);
siRNA-GHSR-1 (si-GHSR-1), 5'-CCTCTACCTGTCCAGCATG-3' (SEQ.ID.NO.37);
siRNA-GHSR-2 (si-GHSR-2), 5'-GCTGGTCATCTTCGTCATC-3' (SEQ.ID.NO.38);
si-RNA-NTSR1-1 (si-NTSR1-1), 5'-GTTCATCAGCGCCATCTGG-3' (SEQ.ID.NO.39);
si-RNA-NTSR1-2 (si-NTSR1-2), 5'-GGTCGTCATACAGGTCAAC-3' (SEQ.ID.NO.40).

The oligonucleotides used for these siRNAs are shown below. Each constructs were prepared by cloning the following double-stranded oligonucleotide into the BbsI site in the psiH1BX3.0 vector. The corresponding nucleotide position relative to the KIF11, GHSR1b, and NTSR1 nucleic acid sequence of SEQ ID NOs:1, 3 and 5 are listed for each oligonucleotide sequence. Each oligonucleotide is a combination of a sense nucleotide sequence and an antisense nucleotide sequence of the target sequence of KIF11, GHSR1b, and NTSR1. The nucleotide sequences of the hairpin loop structure of each siRNAs are also shown bellow. (endonuclease

recognition sites are eliminated from each hairpin loop structure sequence).

KIF11 si1 288-306 (for the target sequence of gttagtgtac gaactggag/ SEQ ID NO:32)
(insert F) Tccc gttagtgtacgaactggag ttcaagaga ctccagttcgtacactaac/SEQ ID NO:54
(insert R) Aaaa gttagtgtacgaactggag tctcttgaa ctccagttcgtacactaac/SEQ ID NO:55
(hairpin) gttagtgtacgaactggag ttcaagaga ctccagttcgtacactaac/SEQ ID NO:56

KIF11 si2 612-630 (for the target sequence of gtgtctctgt tggagatct/ SEQ ID NO:33)
(insert F) Tccc gtgtctctgt tggagatct ttcaagaga agatctccaacagagacac/SEQ ID NO:57
(insert R) Aaaa gtgtctctgt tggagatct tctcttgaa agatctccaacagagacac/SEQ ID NO:58
(hairpin) gtgtctctgt tggagatct ttcaagaga agatctccaacagagacac/SEQ ID NO:59

KIF11 si3 1700-1718 (for the target sequence of gaaggcagtt gaccaacac/ SEQ ID NO:34)
(insert F) Tccc gaaggcagtt gaccaacac ttcaagaga gtgttggtcaactgccttc/SEQ ID NO:60
(insert R) Aaaa gaaggcagtt gaccaacac tctcttgaa gtgttggtcaactgccttc/SEQ ID NO:61
(hairpin) gaaggcagtt gaccaacac ttcaagaga gtgttggtcaactgccttc/SEQ ID NO:62

KIF11 si4 2580-2598 (for the target sequence of gagactgaac agagatgtg/ SEQ ID NO:35)
(insert F) Tccc gagactgaac agagatgtg ttcaagaga cacatctctgttcagtctc/SEQ ID NO:63
(insert R) Aaaa gagactgaac agagatgtg tctcttgaa cacatctctgttcagtctc/SEQ ID NO:64
(hairpin) gagactgaac agagatgtg ttcaagaga cacatctctgttcagtctc/SEQ ID NO:65

KIF11 si5 2695-2713 (for the target sequence of gccaatgttg tgaggcttc/ SEQ ID NO:36)
(insert F) Tccc gccaatgttg tgaggcttc ttcaagaga gaagcctcacaacattggc/SEQ ID NO:66
(insert R) Aaaa gccaatgttg tgaggcttc tctcttgaa gaagcctcacaacattggc/SEQ ID NO:67
(hairpin) gccaatgttg tgaggcttc ttcaagaga gaagcctcacaacattggc/SEQ ID NO:68

GHSR1b si1 237-255 (for the target sequence of cctctacctg tccagcatg/ SEQ ID NO:37)
(insert F) Tccc cctctacctg tccagcatg ttcaagaga catgctggacaggtagagg/SEQ ID NO:69
(insert R) Aaaa cctctacctg tccagcatg tctcttgaa catgctggacaggtagagg/SEQ ID NO:70
(hairpin) cctctacctg tccagcatg ttcaagaga catgctggacaggtagagg/SEQ ID NO:71

GHSR1b si2 483-501 (for the target sequence of gctggtcatc ttcgtcatc/ SEQ ID NO:38)
(insert F) Tccc gctggtcatc ttcgtcatc ttcaagaga gatgacgaagatgaccagc/SEQ ID NO:72
(insert R) Aaaa gctggtcatc ttcgtcatc tctcttgaa gatgacgaagatgaccagc/SEQ ID NO:73
(hairpin) gctggtcatc ttcgtcatc ttcaagaga gatgacgaagatgaccagc/SEQ ID NO:74

NTSR1 si1 933-951 (for the target sequence of gttcatcagc gccatctgg/ SEQ ID NO:39)
(insert F) Tccc gttcatcagc gccatctgg ttcaagaga ccagatggcgctgatgaac/SEQ ID NO:75
(insert R)Aaaa gttcatcagc gccatctgg tctcttgaa ccagatggcgctgatgaac/SEQ ID NO:76
(hairpin) gttcatcagc gccatctgg ttcaagaga ccagatggcgctgatgaac/SEQ ID NO:77

NTSR1 si2 1074-1092 (for the target sequence of ggtcgtcata caggtcaac/ SEQ ID NO:40)
(insert F) Tccc ggtcgtcata caggtcaac ttcaagaga gttgacctgtatgacgacc/SEQ ID NO:78
(insert R) Aaaa ggtcgtcata caggtcaac tctcttgaa gttgacctgtatgacgacc/SEQ ID NO:79
(hairpin) ggtcgtcata caggtcaac ttcaagaga gttgacctgtatgacgacc/SEQ ID NO:80

To validate RNAi system of the present invention, individual control siRNAs (EGFP, Luciferase, and Scramble) were initially confirmed using semiquantitative RT-PCR to decrease the expression of the corresponding target genes that had been transiently transfected into COS-7 cells. Down-regulation of KIF11, GHSR1b, and NTSR1 expression by their respective siRNAs (si-KIF11-1, si-KIF11-2, si-KIF11-3, si-KIF11-4, si-KIF11-5, si-GHSR-1, si-NTSR1-1, and si-NTSR1-2), but not by controls, was confirmed with semiquantitative RT-PCR in the cell lines used for this assay.

(6) Co-immunoprecipitaion and MALDI-TOF mass spectrometry

Human lung cancer cell line LC319 cells were transfected with bilateral-tagged pCAGGS-n3FH (NH2-terminal FLAG, COOH-terminal HA)-KOC1 expression vector or empty vector (mock transfection). Cells were extracted in IP-buffer (0.5% NP-40, 50 mM Tris-HCl, 150 mM NaCl, and protease inhibitor) for 30 min on ice. Extracts were centrifuged at 14,000 rpm for 15 min, and supernatants were subjected to immunoprecipitation using anti-Flag M2 agarose and anti-HA beads (Sigma-Aldrich) for 1-2 hours. The beads were washed six times with IP-buffer, and protein was eluted by boiling the beads in Laemmli sample buffer after removing the final wash fraction. The eluted protein was resolved by SDS-PAGE and stained with silver staining. A 120 kDa-band was extracted by gel extraction, and used for mass spectrometric sequencing using MALDI-TOF mass spectrometry. This analysis identified KIF11 as the 120 kDa product.

To confirm the interaction between KOC1 and KIF11, A549 cells were transiently co-transfected with Flag-tagged KIF11 and myc-tagged KOC1 and the cells were immunoprecipitated with anti-Flag M2 agarose. Subsequently, the cells were immunoblotted with anti-myc antibody (9E10; Santa Cruz). Next, using the same combination of vectors and cells, the cells were immunoprecipitated with anti-myc agarose (SIGMA) and immunoblotted

with anti-Flag M2 antibody.

To further confirm this interaction, A549 cells were transiently co-transfected with Flag-tagged KIF11 and myc-tagged KOC1, and co-localization of FITC-labeled KIF11 and rhodamine-labeled KOC1 mainly in the cytoplasm was detected by immunocytochemical staining using FITC-labeled anti-FLAG antibody and rhodamine-labeled anti-myc antibody, as described below.

(7) Immunocytochemistry

A549 cells grown on coverslips were cultured for 24 hours after passage, and were co-transfected with Flag-tagged KIF11 and myc-tagged KOC1. After 24-hours incubation, the cells were fixed with acetone/methanol (1:1) for 5 min on ice, blocked in CAS BLOCK (ZYMED) for 7 min at RT, and then incubated with rabbit anti-Flag polyclonal antibody (SIGMA) for 1 hour at RT. The fixed cells were washed 3 times with PBS, reacted with anti-rabbit IgG-FITC for 1 hour at RT. Then the cells were blocked again, and incubated with anti-myc antibody (9E10; Santa Cruz) for 1 hour at RT. Finally anti-mouse IgG-rhodamin was applied to the cells for 1 hour at RT. The cells were viewed on a Leica TCS SP2-AOBS confocal microscope.

(8) Ligand-receptor binding assay

To identify direct binding of NMU-25 to its candidate receptors, GHSR1a, GHSR1b and NTSR1, the following experiments were performed. The entire coding region of each receptor gene was amplified by RT-PCR using primers GHSR1a (5'-GGAATTCCATGTGGAACGCGACGCCCAGCGAA-3' (SEQ.ID.NO.41) and 5'-CGCGGATCCGCGTGTATTAATACTAGATTCTGTCCAGGCC-3' (SEQ.ID.NO.42)), GHSR1b (5'-GGAATTCCATGTGGAACGCGACGCCCAGCGAA-3' (SEQ.ID.NO.43) and 5'-CGCGGATCCGCGGAGAGAAGGGAGAAGGCACAGGGA-3' (SEQ.ID.NO.44)), and NTSR1 (5'-GGAATTCCATGCGCCTCAACAGCTCCGCGCCGGGAA-3' (SEQ.ID.NO.45) and 5'-CGCGGATCCGCGGTACAGCGTCTCGCGGGTGGCATTGCT-3' (SEQ.ID.NO.46)). The products were digested with *Eco*R1 and *Bam*H1 and cloned into appropriate sites of p3XFLAG-CMV10 vector (Sigma-Aldrich Co.). COS-7 cells were transfected with GHSR1b or NTSR1 expression plasmids using FuGENE6, as described above. Transfected COS-7 cells were cultured with 0.5 μM rhodamine-labeled NMU-25 peptide (NMU-25-rhodamine: Phoenix Pharmaceuticals. Inc.) for 12 hours, washed five times in PBS(-), and fixed in 4% paraformaldehyde solution for 60 min at room temperature. Then the cells were incubated with antibodies to FLAG-tagged GHSR1a, GHSR1b, or NTSR1 proteins (Sigma-Aldrich Co.), stained with a goat anti-mouse secondary antibody conjugated to FITC (Cappel) and viewed under laser-confocal microscopy (TCS SP2 AOBS: Leica Microsystems). In addition, three

different negative controls were prepared for this assay: 1) non-transfected COS-7 cells without addition of NMU-25-rhodamine; 2) non-transfected COS-7 cells treated with NMU-25-rhodamine; and 3) COS-7 cells transfected with GHSR1a, GHSR1, or NTSR1 without NMU-25-rhodamine. COS-7 cells transfected with a known NMU receptor (NMU1R) served as a positive control for the assay.

To confirm the binding of NMU-25 to the candidate receptors, flow-cytometric analysis was performed using the same series of COS-7 cells. Specifically, COS-7 cells were plated at a density of 1 X 105 cells/100-mm dish and transfected with either GHSR1b, NTSR1, or NMU1R expression vectors; 24 hours after transfection, cells were incubated with 0.5 μM NMU-25-rhodamine for 12 hours, washed, trypsinized, collected in PBS, and washed once more in PBS. The population of cells binding to rhodamine-labeled NMU-25 was determined by flow cytometry.

Results

(1) Identification of KIF11 as a protein interacting with KOC1

LC319 cells transfected with n3FH-pCAGGS-KOC1 vector were extracted and immunoprecipitated with anti-Flag M2 monoclonal antibody, and subsequently immunoprecipitated with anti-HA monoclonal antibody. The protein complex including KOC1 was stained with silver staining on SDS-PAGE gel. A 120 kDa band that was absent in mock transfection was extracted and determined to be KIF11 (NM_004523;SEQ.ID.NO.1) by Mass spectrometric sequencing. (Fig. 1).

(2) Confirmation of interaction between KOC1 and KIF11

The A549 cells co-transfected with Flag-tagged KIF11 and myc-tagged KOC1, the cells transfected with either KIF11 or KOC1, and the non-transfected cells were immunoprecipitated with anti-Flag M2 agarose and subsequently immunoblotted with anti-myc antibody. In contrast, the same series of A549 cells were immunoprecipitated with anti-myc agarose and immunoblotted with anti-Flag M2 antibody. A single band was found only when both constructs were co-transfected (Fig. 2a). Immunocytochemistry showed that FLAG-tagged FITC-labeled KIF11 co-localized in cytoplasm of A549 with myc-tagged rhomamine-labeled KOC1 (Fig. 2b).

(3) KIF11 expression in NSCLC

Validation of KIF11 expression was performed in primary NSCLCs (clinical samples) and lung cancer cell lines. Increased KIF11 expression was confirmed in 12 of 16 NSCLC cases (5 of 8 ADCs and in 7 of 8 SCCs (Fig. 3). In addition, up-regulation of KIF11 were observed in 14 of the 15 NSCLC cell lines (Fig. 3). The expression pattern of KIF11 in NSCLCs was

significantly concordant with that of KOC1.

(4) KIF11 expression in normal human tissues

Northern blotting with KIF11 cDNA as a probe identified 4.5- and 5.5-kb transcripts as very weak bands, only seen in placenta, testis, and bone marrow, among the 23 normal human tissues examined. The expression pattern of KIF11 in normal human tissues was significantly correlated with that of KOC1 (Fig. 4).

(5) Inhibition of growth of NSCLC cells by siRNA against KIF11

Transfection of either siRNA plasmids for KIF11 into A549 (Fig. 5a) or LC319 (data not shown) cells suppressed mRNA expression of the KIF11 in comparison to cells containing any of the three control siRNAs and mock transfection. In accordance with the reduced mRNA expression, A549 and LC319 cells showed significant decreases in cell viability and colony numbers measured by MTT (Fig. 5b) and colony-formation assays (data not shown).

(6) Screening of candidate receptors for NMU in NSCLC

Two known NMU receptors, NMU1R (FM3/GPR66) and NMU2R (FM4) play important roles in energy homeostasis(Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." J. Biol. Chem. 275: 21068-21074 (2000); Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." Nature 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." Peptides 23: 1607-1615 (2002)). NMU1R is present in many peripheral human tissues (Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." J. Biol. Chem. 275: 21068-21074 (2000); Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." Nature 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." Peptides 23: 1607-1615 (2002)), but NMU2R is located only in brain. To investigate whether NMU1R and NMU2R genes were expressed in NSCLCs, expression of these NMU receptors were analyzed in normal human brain and lung, in NSCLC cell lines, and in clinical tissues by semiquantitative RT-PCR experiments. Neither NMU1R nor NMU2R expression was detected in any of the cell lines or clinical samples examined, although NMU1R was expressed in lung and NMU2R in brain (data now shown), suggesting that NMU could be mediating growth of lung-cancer cells through interaction with other receptor(s).

Since NMU2R and NMU1R were originally isolated as homologues of known neuropeptide GPCRs, unidentified NMU receptor(s) were speculated to be members of the same family that would show some degree of homology to NMU1R/NMU2R. Hence, candidate NMU receptors were searched using the BLAST program. The results and their high expression

levels in NSCLCs in the expression profile data of the present inventors indicated GHSR1b (NM_004122;SEQ ID NOs: 3 and 4) and NTSR1 (NM_002531;SEQ ID NOs: 5 and 6) to be good candidates. GHSR has two transcripts, types 1a and 1b. The full-length human type 1a cDNA encodes a predicted polypeptide of 366 amino acids with seven transmembrane domains, a typical feature of G protein-coupled receptors. A single intron divides its open reading frame into two exons encoding transmembrane domains 1-5 and 6-7, thus placing the GHSR1a into the intron-containing class of GPCRs. Type 1b is a non-spliced mRNA variant transcribed from a single exon that encodes a polypeptide of 289 amino acids with five transmembrane domains. The semiquantitative RT-PCR analysis using specific primers for each variant indicated that GHSR1a was not expressed in NSCLCs. On the other hand, GHSR1b and NTSR1 were expressed at a relatively high level in some NSCLC cell lines, but not at all in normal lung (Fig. 7). The GHSR1b product has 46% homology to NMU1R, and NTSR1 encodes 418 amino acids with 47% homology to NMU1R.

(7) Identification of candidate receptors for NMU in NSCLC

To confirm direct interaction between NMU and GHSR1b/NTSR1, COS-7 cells were transiently transfected with plasmids designed to express FLAG-tagged GHSR1b or NTSR1, and cultured in the presence of rhodamine-labeled NMU-25. Then the localization of FLAG-tagged GHSR1b/NTSR1 and NMU-25-rhodamine in the cells were examined using anti-FLAG antibodies conjugated to FITC, and found that NMU-25 and either of both receptors were located together on the cell membrane (Fig. 8a). Co-localization of NMU-25 with these receptors was not observed in control assays involving either of the following ligand/cell combinations: 1) NMU-25-rhodamine incubated with COS-7 cells that were not transfected with either of the receptor plasmids; 2) non-transfected COS-7 cells incubated without NMU-25-rhodamine; and 3) COS-7 cells transfected with either of the receptor plasmids, but incubated without NMU-25-rhodamine. The result was confirmed by flow cytometry, which revealed binding of rhodamine-labeled NMU-25 to the surface of COS-7 cells that expressed either of the two receptors (Fig. 8b).

(8) GHSR1b expression in normal human tissues

As the expression of GHSR1b in normal human tissues was not precisely reported at the time, the distribution of GHSR1b was determined using human multiple tissue Northern-blot. Northern blotting with GHSR1b cDNA as a probe identified a 0.9-kb transcript as a very weak signal band in comparison with a 1.1-kb transcript GHSR1a, seen in the heart, liver, skeletal muscle, pancreas, and stomach, among the 23 normal human tissues examined (Fig. 9).

(9) Inhibition of growth of NSCLC cells by siRNA against GHSR/NTSR1

Furthermore, the biological significance of the NMU-receptor interaction in pulmonary carcinogenesis was examined using plasmids designed to express siRNA against GHSR or NTSR1 (si-GHSR-1, si-NTSR1-1, and si-NTSR1-2). Transfection of either of these plasmids into A549 or LC319 cells suppressed expression of the endogenous receptor in comparison to cells containing any of the three control siRNAs (Fig. 10a). In accordance with the reduced expression of the receptors, A549 and LC319 cells showed significant decreases in cell viability (Fig. 10b) and numbers of colonies (data not shown). These results strongly supported the possibility that NMU, by interaction with GHSR1b and NTSR1, might play a very significant role in development/progression of NSCLC.

Industrial Applicability

The expression of human genes KIF11, GHSR1b and NTSR1 are markedly elevated in non-small cell lung cancer (NSCLC) as compared to normal lung tissues. Accordingly, these genes may serve as diagnostic markers of NSCLC and the proteins encoded thereby may be used in diagnostic assays of NSCLC.

The present inventors have also shown that the expression of KIF11, GHSR1b or NTSR1 promotes cell growth whereas cell growth is suppressed by small interfering RNAs corresponding to KIF11, GHSR1b or NTSR1 gene. These findings suggest that each of KIF11, GHSR1b and NTSR1 proteins stimulate oncogenic activity. Thus, each of these oncoproteins is a useful target for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of KIF11, GHSR1b or NTSR1, or prevent its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of NSCLC. Examples of such agents include antisense oligonucleotides, small interfering RNAs, and ribozymes against the KIF11, GHSR1b or NTSR1 gene, and antibodies that recognize KIF11, GHSR1b or NTSR1 polypeptide.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

What is claimed is:

1. A method of diagnosing non-small cell lung cancer (NSCLC) or a predisposition to developing non-small cell lung cancer in a subject, comprising determining the expression level of a non-small cell lung cancer-associated gene in a biological sample derived from the subject, wherein an increase of said expression level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing NSCLC, wherein said NSCLC-associated gene is selected from the group consisting of KIF11, GHSR1b, and NTSR1.

2. The method of claim 1, wherein said increase is at least 10% greater than said normal control level.

3. The method of claim 1, wherein said method further comprises determining the expression level of a plurality of NSCLC-associated genes.

4. The method of claim 1, wherein said expression level is determined by any one method select from the group consisting of:
(1) detecting mRNA of the NSCLC-associated gene;
(2) detecting protein encoded by the NSCLC-associated gene; and
(3) detecting the biological activity of protein encoded by the NSCLC-associated gene.

5. The method of claim 1, wherein said expression level is determined by detecting hybridization of an NSCLC-associated gene probe to a gene transcript of said patient-derived biological sample.

6. The method of claim 5, wherein said hybridization step is carried out on a DNA array.

7. The method of claim 1, wherein said biological sample comprises sputum or blood.

8. A NSCLC reference expression profile, comprising a gene expression pattern of two or more genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.

9. A kit comprising two or more detection reagents which detect the expression of one or more genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.

10. An array comprising two or more polynucleotides which bind to one or more genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.

11. A method of identifying a compound that inhibits the expression level of an NSCLC-associated gene, comprising the steps of:
(1) contacting a test cell expressing said NSCLC-associated gene with a test compound;

(2) detecting the expression level of said NSCLC-associated gene; and

(3) determining the compound that suppresses said expression level compared to a normal control level of said gene as an inhibitor of said NSCLC-associated gene

wherein said NSCLC-associated gene is selected from the group consisting of KIF11, GHSR1b, and NTSR1.

12. The method of claim 11, wherein said test cell is NSCLC cell.

13. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

(1) contacting a test compound with a polypeptide selected from the group consisting of KIF11, GHSR1b, and NTSR1;

(2) detecting the binding activity between the polypeptide and the test compound; and

(3) selecting a compound that binds to the polypeptide.

14. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

(a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of KIF11, GHSR1b, and NTSR1;

(b) detecting the biological activity of the polypeptide of step (a); and

(c) selecting a compound that suppresses the biological activity of the polypeptide selected from the group consisting of KIF11, GHSR1b, and NTSR1 in comparison with the biological activity detected in the absence of the test compound.

15. The method of claim 14, wherein said biological activity is cell proliferative activity.

16. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

(1) contacting a test compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of KIF11, GHSR1b, and NTSR1; and

(2) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.

17. The method of claim 16, wherein said cell is NSCLC cell.

18. A method of screening for compound for treating or preventing NSCLC, said method comprising the steps of:

(1) contacting a test compound with a cell into which a vector comprising the

transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of KIF11, GHSR1b, and NTSR1;

(2) measuring the activity of said reporter gene; and

(3) selecting a compound that reduces the expression level of said reporter gene, as compared to a control.

19. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

(1) contacting a KIF11 polypeptide or functional equivalent thereof with KOC1 in the presence of a test compound;

(2) detecting the binding between the polypeptide and KOC1; and

(3) selecting the test compound that inhibits the binding between the polypeptide and KOC1.

20. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

(1) contacting a GHSR1b or NTSR1 polypeptide, or functional equivalent thereof with NMU in the existence of a test compound;

(2) detecting the binding between the polypeptide and NMU; and

(3) selecting the test compound that inhibits the binding between the polypeptide and NMU.

21. The method of claim 20, wherein the polypeptide is expressed on the surface of a living cell.

22. The method of claim 21, wherein the binding between the polypeptide and NMU is detected by any one method select from the group consisting of:

(1) detecting the concentration of calcium or cAMP in the cell;

(2) detecting the activation of the polypeptide;

(3) detecting the interaction between the polypeptide and G-protein;

(4) detecting the activation of phospholipase C or its down stream pathway;

(5) detecting the activation of protein kinase cascade leading to activation of several kinases including Raf, MEK, ERKs, and protein kinase D (PKD);

(6) detecting the activation of a member of Src/Tec/Bmx-family of tyrosine kinases;

(7) detecting the activation of a member of the Ras and Rho family, regulation of a member of the JNK members of MAP families, or the reorganization of the actin cytoskeleton;

(8) detecting the activation of any signal complex mediated by the polypeptide activation;

(9) detecting the change in subcellular localization of the polypeptide including the ligand-induced internalization/endocytosis of the polypeptide;

(10) detecting the activation of any transcription factor downstream of the polypeptides or the activation of their downstream gene; and

(11) detecting cell proliferation, transformation, or any other oncogenic phenotype.

23. A method of treating or preventing NSCLC in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence of a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

24. A method of treating or preventing NSCLC in a subject comprising administering to said subject an siRNA composition comprising an siRNA, wherein said composition reduces the expression of a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

25. The method of claim 24, wherein the siRNA is a sense strand comprising the nucleotide sequence selected from the group of SEQ ID NOs: 32, 33, 34, 37, 39, and 40, as the target sequence.

26. The method of claim 25, wherein the siRNA has the general formula

5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A].

27. A method for treating or preventing NSCLC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a polypeptide encoded by a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

28. A method of treating or preventing NSCLC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

29. A method for treating or preventing NSCLC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claim 13

to claim 22.

30. A double-stranded molecule comprising a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a KIF11, GHSR1b, or NTSR1 target sequence, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing a KIF11, GHSR1b, or NTSR1 gene, inhibits expression of said gene.

31. The double-stranded molecule of claim 30, wherein said KIF11, GHSR1b, or NTSR1 target sequence comprises at least about 10 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3 and 5.

32. The double-stranded molecule of claim 31, wherein said KIF11, GHSR1b, or NTSR1 target sequence comprises from about 19 to about 25 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3 and 5.

33. The double-stranded molecule of claim 32, wherein said KIF11, GHSR1b, or NTSR1 target sequence is selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40.

34. The double-stranded molecule of claim 30, wherein said double-stranded molecule is a single ribonucleotide transcript comprising the sense strand and the antisense strand linked via a single-stranded ribonucleotide sequence.

35. The double-stranded molecule of claim 30, wherein the double-stranded molecule is an oligonucleotide of less than about 100 nucleotides in length.

36. The double-stranded molecule of claim 35, wherein the double-stranded molecule is an oligonucleotide of less than about 75 nucleotides in length.

37. The double-stranded molecule of claim 36, wherein the double-stranded molecule is an oligonucleotide of less than about 50 nucleotides in length.

38. The double-stranded molecule of claim 37, wherein the double-stranded molecule is an oligonucleotide of less than about 25 nucleotides in length.

39. The double-stranded polynucleotide of claim 38, wherein the double stranded molecule is an oligonucleotide of between about 19 and about 25 nucleotides in length.

40. A vector encoding the double-stranded molecule of claim 30.

41. The vector of claim 40, wherein the vector encodes a transcript having a secondary structure and comprises the sense strand and the antisense strand.

42. The vector of claim 41, wherein the transcript further comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand.

43. A vector comprising a polynucleotide comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid, wherein said sense strand nucleic acid comprises nucleotide sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40, and said antisense strand nucleic acid consists of a sequence complementary to the sense strand.

44 The vector of claim 43, wherein said polynucleotide has the general formula
5'-[A]-[B]-[A']-3',
wherein [A] is a nucleotide sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40; [B] is a nucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a nucleotide sequence complementary to [A].

45. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide against a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

46. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of an siRNA against a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

47. The composition of claim 46, wherein the siRNA comprises a sense strand comprising the nucleotide sequence of SEQ ID NO: 32 to SEQ ID NO: 40, as the target sequence.

48. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a polypeptide encoded by a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

49. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claim 13 to claim 22 as an active ingredient, and a pharmaceutically acceptable carrier.

ABSTRACT

Disclosed are methods for detecting non-small cell lung cancer (NSCLC) using differentially expressed genes KIF11, GHSR1b, and NTSR1. Also disclosed are methods of identifying compounds for treating and preventing NSCLC, based on the interaction between KOC1 and KIF11, or NMU and GHSR1b or NTSR1.

Fig. 1

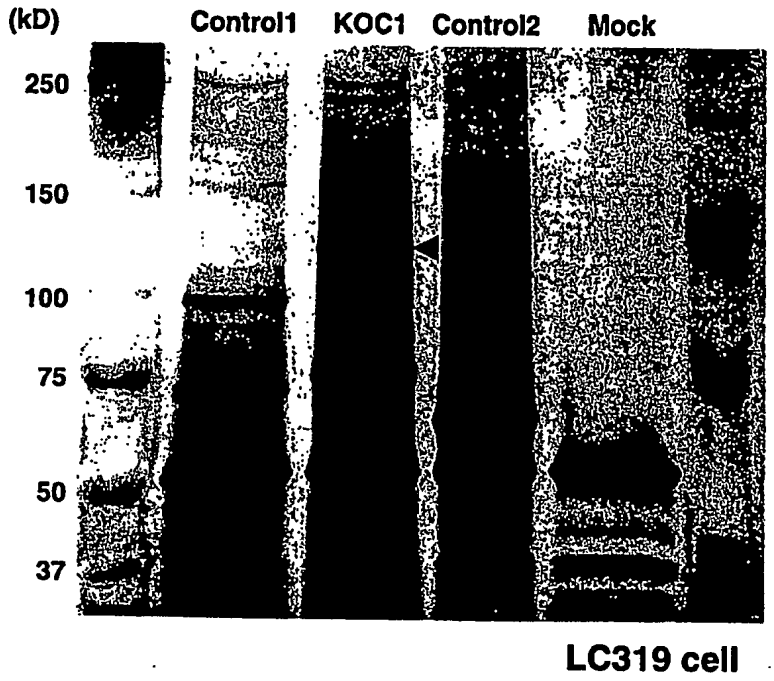

Fig. 2

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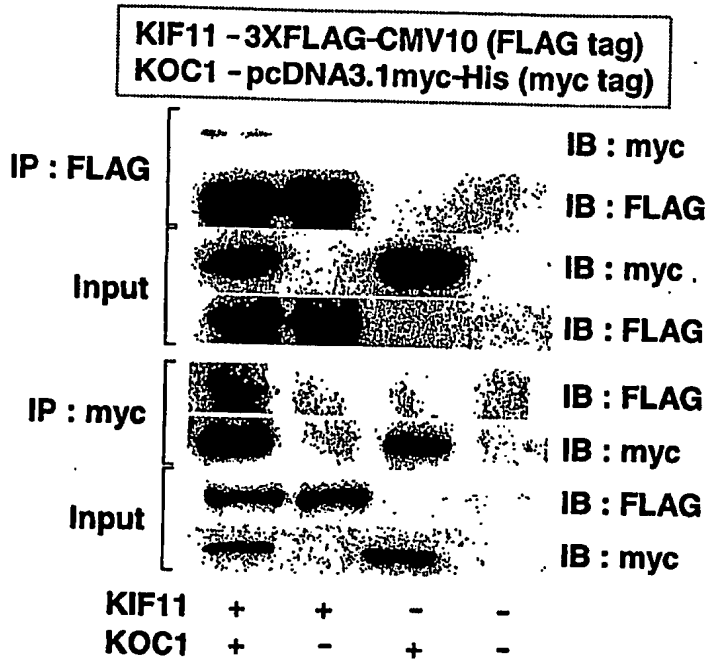

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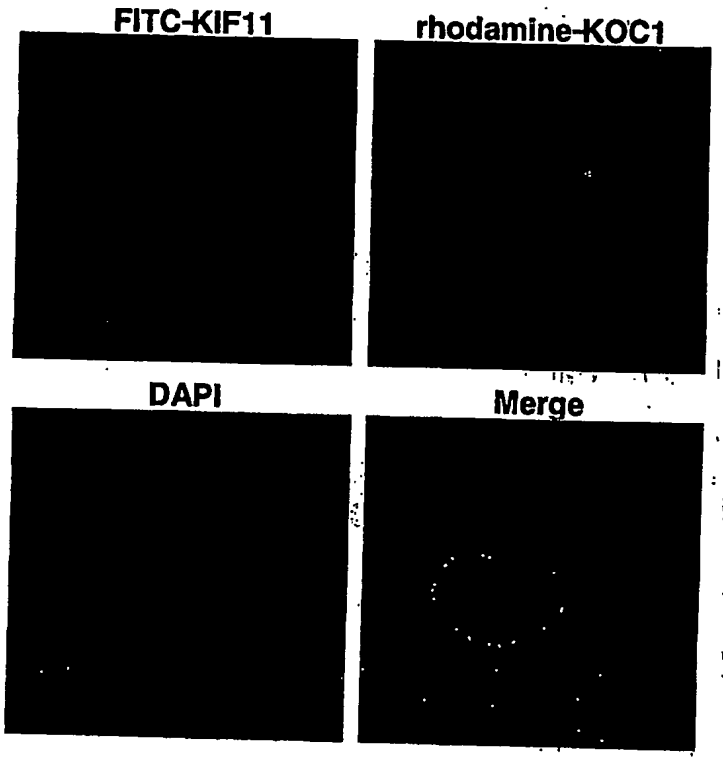

Fig. 3

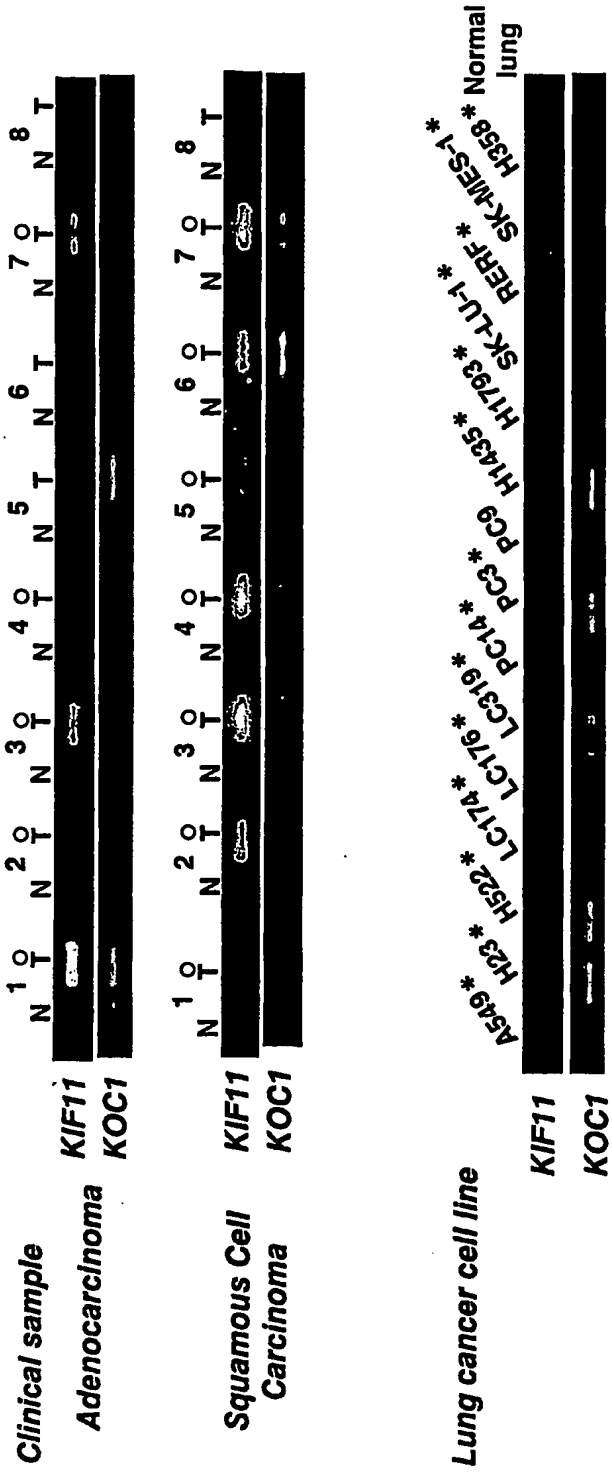

Fig. 4

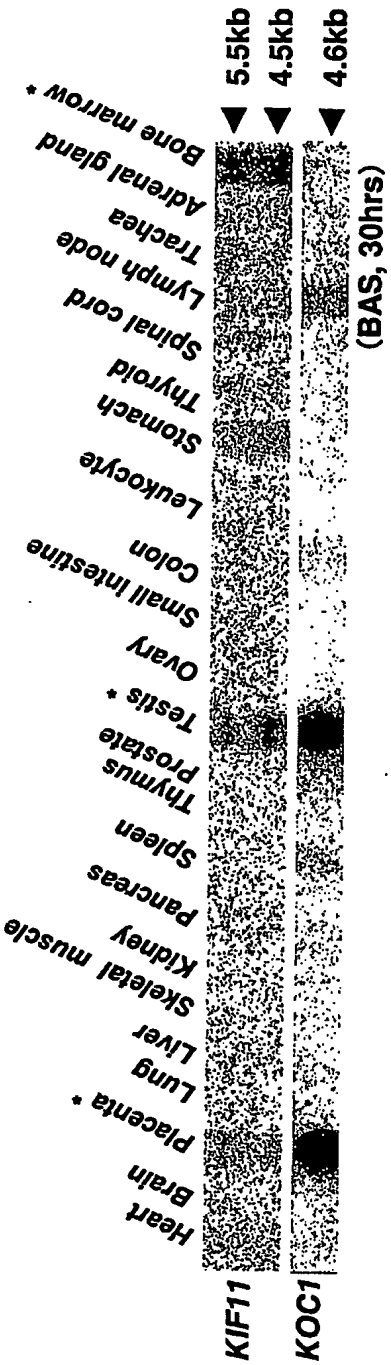

Fig. 5

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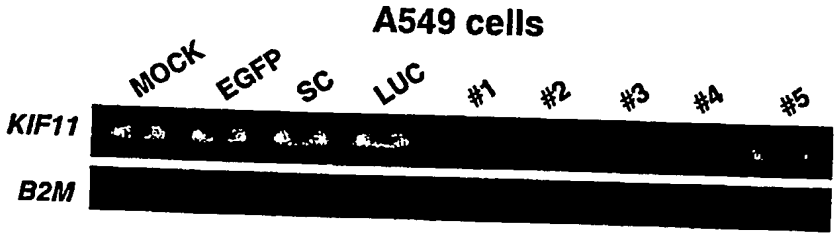

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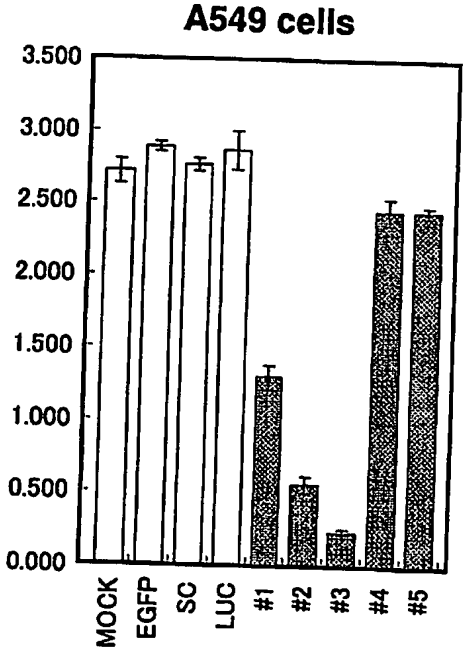

Fig. 6

a

b

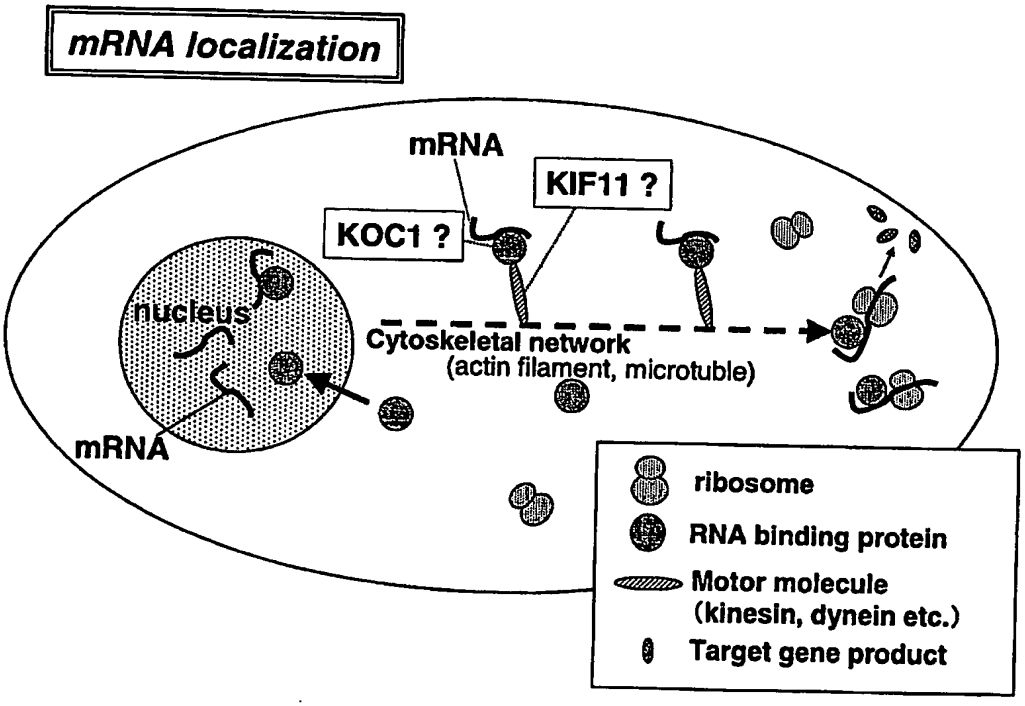

Fig. 7

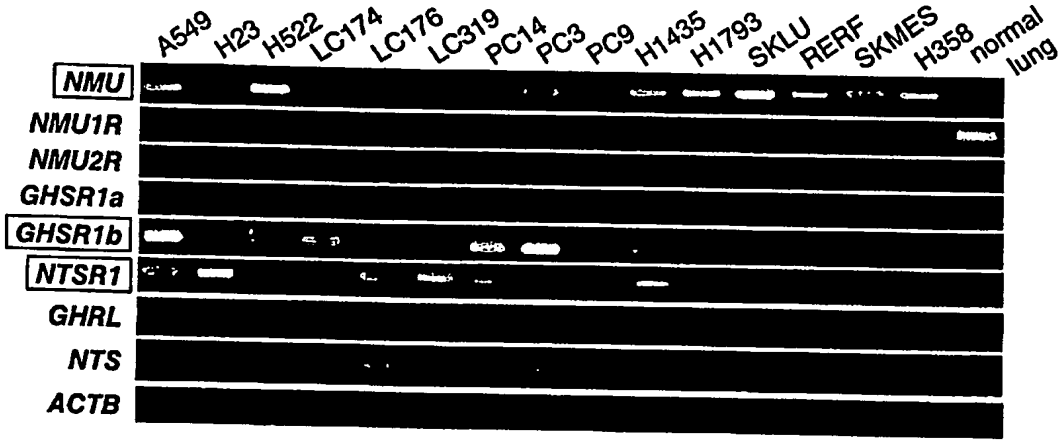

Fig. 8

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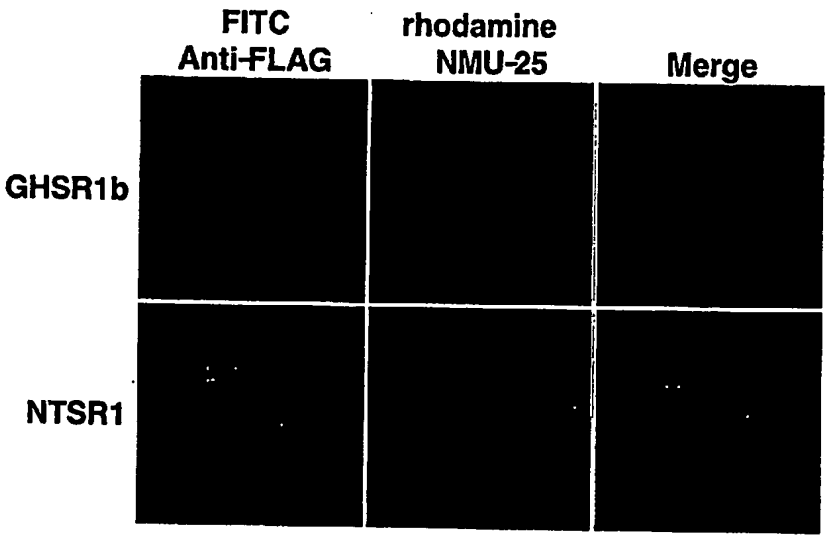

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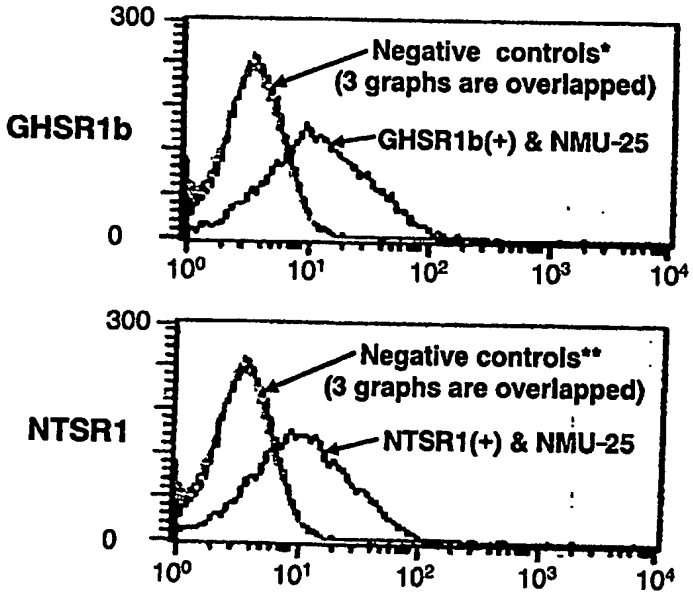


*Three control assays used for this study:
1. COS-7 cells (no receptor) only
2. No receptor & NMU-25
3. GHSR1b(+)-COS-7 cells only

**Three control assays used for this study:
1. COS-7 cells (no receptor) only
2. No receptor & NMU-25
3. NTSR1(+)-COS-7 cells only

Fig. 9

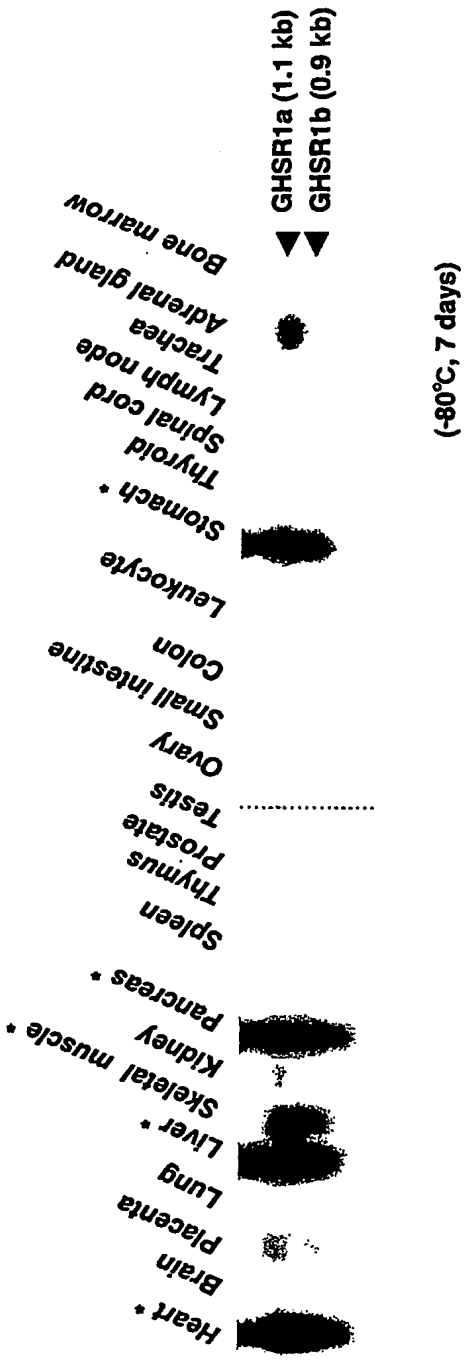
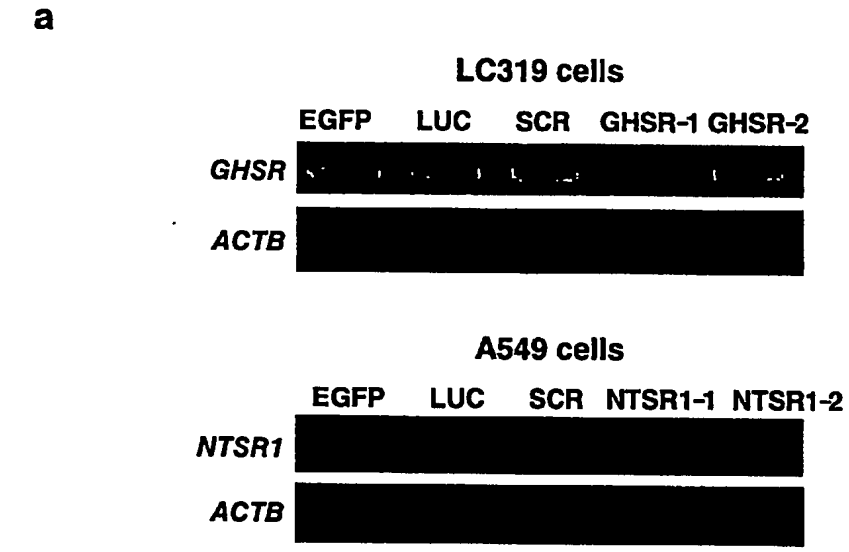


Fig. 10



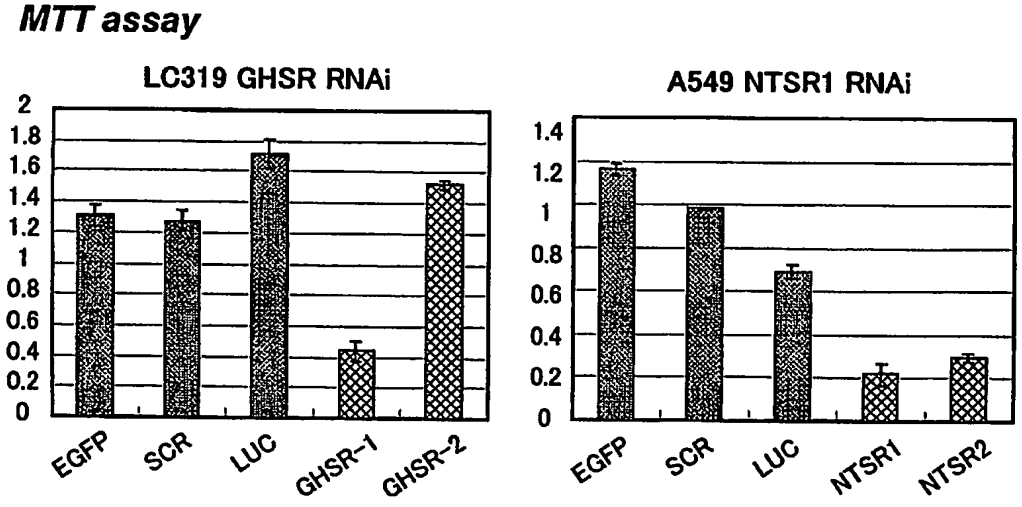


Fig. 11

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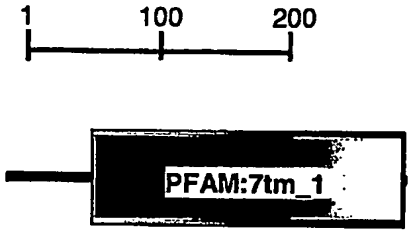

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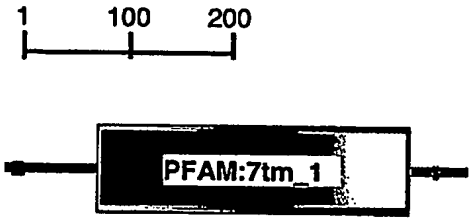

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<110> Nakamura, Yusuke
Daigo, Yataro
Nakatsuru, Shuichi

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Arg Thr Glu Pro Arg Glu His Leu Leu Asp Gln Leu Lys Arg Lys Gln
940                 945                 950                 955

cct gag ctg tta atg atg cta aac tgt tca gaa aac aac aaa gaa gag      3053
Pro Glu Leu Leu Met Met Leu Asn Cys Ser Glu Asn Asn Lys Glu Glu
                960                 965                 970

aca att ccg gat gtg gat gta gaa gag gca gtt ctg ggg cag tat act      3101
Thr Ile Pro Asp Val Asp Val Glu Glu Ala Val Leu Gly Gln Tyr Thr
            975                 980                 985

gaa gaa cct cta agt caa gag cca tct gta gat gct ggt gtg gat tgt      3149
Glu Glu Pro Leu Ser Gln Glu Pro Ser Val Asp Ala Gly Val Asp Cys
        990                 995                 1000

tca tca att ggc ggg gtt cca ttt ttc cag cat aaa aaa tca cat          3194
Ser Ser Ile Gly Gly Val Pro Phe Phe Gln His Lys Lys Ser His
    1005                1010                1015

gga aaa gac aaa gaa aac aga ggc att aac aca ctg gag agg tct          3239
Gly Lys Asp Lys Glu Asn Arg Gly Ile Asn Thr Leu Glu Arg Ser
    1020                1025                1030

aaa gtg gaa gaa act aca gag cac ttg gtt aca aag agc aga tta          3284
Lys Val Glu Glu Thr Thr Glu His Leu Val Thr Lys Ser Arg Leu
    1035                1040                1045

cct ctg cga gcc cag atc aac ctt taa ttcacttggg ggttggcaat            3331
Pro Leu Arg Ala Gln Ile Asn Leu
    1050                1055

tttatttta aagaaaactt aaaaataaaa cctgaaaccc cagaacttga gccttgtgta    3391
tagattttaa aagaatatat atatcagccg ggcgcggtgg ctcatgcctg taatcccagc    3451
actttgggag gctgaggcgg gtggattgct tgagcccagg agtttgagac cagcctggcc    3511
```

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aacgtggcaa aacctcgtct ctgttaaaaa ttagccgggc gtggtggcac actcctgtaa   3571
tcccagctac tggggaggct gaggcacgag aatcacttga acccaggaag cggggttgca   3631
gtgagccaaa ggtacaccac tacactccag cctgggcaac agagcaagac tcggtctcaa   3691
aaacaaaatt taaaaaagat ataaggcagt actgtaaatt cagttgaatt ttgatatcta   3751
cccatttttc tgtcatccct atagttcact ttgtattaaa ttgggtttca tttgggattt   3811
gcaatgtaaa tacgtatttc tagttttcat ataaagtagt tcttttataa caaatgaaaa   3871
gtatttttct tgtatattat taagtaatga atatataaga actgtactct tctcagcttg   3931
agcttaacat aggtaaatat caccaacatc tgtccttaga aaggaccatc tcatgttttt   3991
tttcttgcta tgacttgtgt attttcttgc atcctcccta gacttcccta tttcgctttc   4051
tcctcggctc actttctccc tttttatttt tcaccaaacc atttgtagag ctacaaaacc   4111
tatcctttct tattttcagt agtcagaatt ttatctagaa atcttttaac accttttag    4171
tggttatttc taaaatcact gtcaacaata aatctaaccc tagttgtatc cctcctttaa   4231
gtatttaaaa cttgttgccc caaatgtgaa agcatttaat tcctttaaga ggcctaactc   4291
attcaccctg acagagttca caaaaagccc actttagagt atacattgct attatgggag   4351
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aagctcacaa tgatttaagg actgtttgaa acttccaatt atgtctataa tttatattct   4471
tttgtttaca tgatgaaact ttttgttgtt gcttgtttgt atataataca atgtgtacat   4531
gtatcttttt ctcgattcaa atcttaaccc ttaggactct ggtatttttg atctggcaac   4591
catatttctg gaagttgaga tgtttcagct tgaagaacca aaacagaagg aatatgtaca   4651
aagaataaat tttctgctca cgatgagttt agtgtgtaaa gtttagagac atctgacttt   4711
gatagctaaa ttaaaccaaa ccctattgaa gaattgaata tatgctaott caagaaacta   4771
aattgatctc gtagaattat cttaataaaa taatggctat aatttctctg caaaatcaga   4831
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aatgtggttt ctacatt                                                  4908
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<210> 2
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<212> PRT
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Met Ala Ser Gln Pro Asn Ser Ser Ala Lys Lys Lys Glu Glu Lys Gly
1               5                  10                  15

Lys Asn Ile Gln Val Val Val Arg Cys Arg Pro Phe Asn Leu Ala Glu
            20                  25                  30

Arg Lys Ala Ser Ala His Ser Ile Val Glu Cys Asp Pro Val Arg Lys
        35                  40                  45

Glu Val Ser Val Arg Thr Gly Gly Leu Ala Asp Lys Ser Ser Arg Lys
    50                  55                  60

Thr Tyr Thr Phe Asp Met Val Phe Gly Ala Ser Thr Lys Gln Ile Asp
65                  70                  75                  80

Val Tyr Arg Ser Val Val Cys Pro Ile Leu Asp Glu Val Ile Met Gly
                85                  90                  95

Tyr Asn Cys Thr Ile Phe Ala Tyr Gly Gln Thr Gly Thr Gly Lys Thr
            100                 105                 110

Phe Thr Met Glu Gly Glu Arg Ser Pro Asn Glu Glu Tyr Thr Trp Glu
        115                 120                 125

Glu Asp Pro Leu Ala Gly Ile Ile Pro Arg Thr Leu His Gln Ile Phe
    130                 135                 140

Glu Lys Leu Thr Asp Asn Gly Thr Glu Phe Ser Val Lys Val Ser Leu
```

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145                 150                 155                 160

Leu Glu Ile Tyr Asn Glu Glu Leu Phe Asp Leu Leu Asn Pro Ser Ser
                165                 170                 175

Asp Val Ser Glu Arg Leu Gln Met Phe Asp Asp Pro Arg Asn Lys Arg
            180                 185                 190

Gly Val Ile Ile Lys Gly Leu Glu Glu Ile Thr Val His Asn Lys Asp
        195                 200                 205

Glu Val Tyr Gln Ile Leu Glu Lys Gly Ala Ala Lys Arg Thr Thr Ala
    210                 215                 220

Ala Thr Leu Met Asn Ala Tyr Ser Ser Arg Ser His Ser Val Phe Ser
225                 230                 235                 240

Val Thr Ile His Met Lys Glu Thr Thr Ile Asp Gly Glu Glu Leu Val
                245                 250                 255

Lys Ile Gly Lys Leu Asn Leu Val Asp Leu Ala Gly Ser Glu Asn Ile
            260                 265                 270

Gly Arg Ser Gly Ala Val Asp Lys Arg Ala Arg Glu Ala Gly Asn Ile
        275                 280                 285

Asn Gln Ser Leu Leu Thr Leu Gly Arg Val Ile Thr Ala Leu Val Glu
    290                 295                 300

Arg Thr Pro His Val Pro Tyr Arg Glu Ser Lys Leu Thr Arg Ile Leu
305                 310                 315                 320

Gln Asp Ser Leu Gly Gly Arg Thr Arg Thr Ser Ile Ile Ala Thr Ile
                325                 330                 335

Ser Pro Ala Ser Leu Asn Leu Glu Glu Thr Leu Ser Thr Leu Glu Tyr
            340                 345                 350

Ala His Arg Ala Lys Asn Ile Leu Asn Lys Pro Glu Val Asn Gln Lys
        355                 360                 365

Leu Thr Lys Lys Ala Leu Ile Lys Glu Tyr Thr Glu Glu Ile Glu Arg
    370                 375                 380

Leu Lys Arg Asp Leu Ala Ala Ala Arg Glu Lys Asn Gly Val Tyr Ile
385                 390                 395                 400

Ser Glu Glu Asn Phe Arg Val Met Ser Gly Lys Leu Thr Val Gln Glu
                405                 410                 415

Glu Gln Ile Val Glu Leu Ile Glu Lys Ile Gly Ala Val Glu Glu Glu
            420                 425                 430

Leu Asn Arg Val Thr Glu Leu Phe Met Asp Asn Lys Asn Glu Leu Asp
        435                 440                 445

Gln Cys Lys Ser Asp Leu Gln Asn Lys Thr Gln Glu Leu Glu Thr Thr
    450                 455                 460

Gln Lys His Leu Gln Glu Thr Lys Leu Gln Leu Val Lys Glu Glu Tyr
```

```
465                 470                 475                 480

Ile Thr Ser Ala Leu Glu Ser Thr Glu Glu Lys Leu His Asp Ala Ala
                485                 490                 495

Ser Lys Leu Leu Asn Thr Val Glu Glu Thr Thr Lys Asp Val Ser Gly
            500                 505                 510

Leu His Ser Lys Leu Asp Arg Lys Lys Ala Val Asp Gln His Asn Ala
        515                 520                 525

Glu Ala Gln Asp Ile Phe Gly Lys Asn Leu Asn Ser Leu Phe Asn Asn
    530                 535                 540

Met Glu Glu Leu Ile Lys Asp Gly Ser Ser Lys Gln Lys Ala Met Leu
545                 550                 555                 560

Glu Val His Lys Thr Leu Phe Gly Asn Leu Leu Ser Ser Ser Val Ser
                565                 570                 575

Ala Leu Asp Thr Ile Thr Thr Val Ala Leu Gly Ser Leu Thr Ser Ile
            580                 585                 590

Pro Glu Asn Val Ser Thr His Val Ser Gln Ile Phe Asn Met Ile Leu
        595                 600                 605

Lys Glu Gln Ser Leu Ala Ala Glu Ser Lys Thr Val Leu Gln Glu Leu
    610                 615                 620

Ile Asn Val Leu Lys Thr Asp Leu Leu Ser Ser Leu Glu Met Ile Leu
625                 630                 635                 640

Ser Pro Thr Val Val Ser Ile Leu Lys Ile Asn Ser Gln Leu Lys His
                645                 650                 655

Ile Phe Lys Thr Ser Leu Thr Val Ala Asp Lys Ile Glu Asp Gln Lys
            660                 665                 670

Lys Glu Leu Asp Gly Phe Leu Ser Ile Leu Cys Asn Asn Leu His Glu
        675                 680                 685

Leu Gln Glu Asn Thr Ile Cys Ser Leu Val Glu Ser Gln Lys Gln Cys
    690                 695                 700

Gly Asn Leu Thr Glu Asp Leu Lys Thr Ile Lys Gln Thr His Ser Gln
705                 710                 715                 720

Glu Leu Cys Lys Leu Met Asn Leu Trp Thr Glu Arg Phe Cys Ala Leu
                725                 730                 735

Glu Glu Lys Cys Glu Asn Ile Gln Lys Pro Leu Ser Ser Val Gln Glu
            740                 745                 750

Asn Ile Gln Gln Lys Ser Lys Asp Ile Val Asn Lys Met Thr Phe His
        755                 760                 765

Ser Gln Lys Phe Cys Ala Asp Ser Asp Gly Phe Ser Gln Glu Leu Arg
    770                 775                 780

Asn Phe Asn Gln Glu Gly Thr Lys Leu Val Glu Glu Ser Val Lys His
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785 790 795 800

Ser Asp Lys Leu Asn Gly Asn Leu Glu Lys Ile Ser Gln Glu Thr Glu
805 810 815

Gln Arg Cys Glu Ser Leu Asn Thr Arg Thr Val Tyr Phe Ser Glu Gln
820 825 830

Trp Val Ser Ser Leu Asn Glu Arg Glu Gln Glu Leu His Asn Leu Leu
835 840 845

Glu Val Val Ser Gln Cys Cys Glu Ala Ser Ser Ser Asp Ile Thr Glu
850 855 860

Lys Ser Asp Gly Arg Lys Ala Ala His Glu Lys Gln His Asn Ile Phe
865 870 875 880

Leu Asp Gln Met Thr Ile Asp Glu Asp Lys Leu Ile Ala Gln Asn Leu
885 890 895

Glu Leu Asn Glu Thr Ile Lys Ile Gly Leu Thr Lys Leu Asn Cys Phe
900 905 910

Leu Glu Gln Asp Leu Lys Leu Asp Ile Pro Thr Gly Thr Thr Pro Gln
915 920 925

Arg Lys Ser Tyr Leu Tyr Pro Ser Thr Leu Val Arg Thr Glu Pro Arg
930 935 940

Glu His Leu Leu Asp Gln Leu Lys Arg Lys Gln Pro Glu Leu Leu Met
945 950 955 960

Met Leu Asn Cys Ser Glu Asn Asn Lys Glu Glu Thr Ile Pro Asp Val
965 970 975

Asp Val Glu Glu Ala Val Leu Gly Gln Tyr Thr Glu Glu Pro Leu Ser
980 985 990

Gln Glu Pro Ser Val Asp Ala Gly Val Asp Cys Ser Ser Ile Gly Gly
995 1000 1005

Val Pro Phe Phe Gln His Lys Lys Ser His Gly Lys Asp Lys Glu
1010 1015 1020

Asn Arg Gly Ile Asn Thr Leu Glu Arg Ser Lys Val Glu Glu Thr
1025 1030 1035

Thr Glu His Leu Val Thr Lys Ser Arg Leu Pro Leu Arg Ala Gln
1040 1045 1050

Ile Asn Leu
1055

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<223>

<400> 3

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atg tgg aac gcg acg ccc agc gaa gag ccg ggg ttc aac ctc aca ctg      48
Met Trp Asn Ala Thr Pro Ser Glu Glu Pro Gly Phe Asn Leu Thr Leu
1               5                  10                  15

gcc gac ctg gac tgg gat gct tcc ccc ggc aac gac tcg ctg ggc gac      96
Ala Asp Leu Asp Trp Asp Ala Ser Pro Gly Asn Asp Ser Leu Gly Asp
            20                  25                  30

gag ctg ctg cag ctc ttc ccc gcg ccg ctg ctg gcg ggc gtc aca gcc     144
Glu Leu Leu Gln Leu Phe Pro Ala Pro Leu Leu Ala Gly Val Thr Ala
        35                  40                  45

acc tgc gtg gca ctc ttc gtg gtg ggt atc gct ggc aac ctg ctc acc     192
Thr Cys Val Ala Leu Phe Val Val Gly Ile Ala Gly Asn Leu Leu Thr
    50                  55                  60

atg ctg gtg gtg tcg cgc ttc cgc gag ctg cgc acc acc acc aac ctc     240
Met Leu Val Val Ser Arg Phe Arg Glu Leu Arg Thr Thr Thr Asn Leu
65                  70                  75                  80

tac ctg tcc agc atg gcc ttc tcc gat ctg ctc atc ttc ctc tgc atg     288
Tyr Leu Ser Ser Met Ala Phe Ser Asp Leu Leu Ile Phe Leu Cys Met
                85                  90                  95

ccc ctg gac ctc gtt cgc ctc tgg cag tac cgg ccc tgg aac ttc ggc     336
Pro Leu Asp Leu Val Arg Leu Trp Gln Tyr Arg Pro Trp Asn Phe Gly
            100                 105                 110

gac ctc ctc tgc aaa ctc ttc caa ttc gtc agt gag agc tgc acc tac     384
Asp Leu Leu Cys Lys Leu Phe Gln Phe Val Ser Glu Ser Cys Thr Tyr
        115                 120                 125

gcc acg gtg ctc acc atc aca gcg ctg agc gtc gag cgc tac ttc gcc     432
Ala Thr Val Leu Thr Ile Thr Ala Leu Ser Val Glu Arg Tyr Phe Ala
    130                 135                 140

atc tgc ttc cca ctc cgg gcc aag gtg gtg gtc acc aag ggg cgg gtg     480
Ile Cys Phe Pro Leu Arg Ala Lys Val Val Val Thr Lys Gly Arg Val
145                 150                 155                 160

aag ctg gtc atc ttc gtc atc tgg gcc gtg gcc ttc tgc agc gcc ggg     528
Lys Leu Val Ile Phe Val Ile Trp Ala Val Ala Phe Cys Ser Ala Gly
                165                 170                 175

ccc atc ttc gtg cta gtc ggg gtg gag cac gag aac ggc acc gac cct     576
Pro Ile Phe Val Leu Val Gly Val Glu His Glu Asn Gly Thr Asp Pro
            180                 185                 190

tgg gac acc aac gag tgc cgc ccc acc gag ttt gcg gtg cgc tct gga     624
Trp Asp Thr Asn Glu Cys Arg Pro Thr Glu Phe Ala Val Arg Ser Gly
        195                 200                 205

ctg ctc acg gtc atg gtg tgg gtg tcc agc atc ttc ttc ttc ctt cct     672
Leu Leu Thr Val Met Val Trp Val Ser Ser Ile Phe Phe Phe Leu Pro
    210                 215                 220
```

```
gtc ttc tgt ctc acg gtc ctc tac agt ctc atc ggc agg aag ctg tgg      720
Val Phe Cys Leu Thr Val Leu Tyr Ser Leu Ile Gly Arg Lys Leu Trp
225                 230                 235                 240

cgg agg agg cgc ggc gat gct gtc gtg ggt gcc tcg ctc agg gac cag      768
Arg Arg Arg Arg Gly Asp Ala Val Val Gly Ala Ser Leu Arg Asp Gln
                245                 250                 255

aac cac aag caa acc gtg aaa atg ctg ggt ggg tct cag cgc gcg ctc      816
Asn His Lys Gln Thr Val Lys Met Leu Gly Gly Ser Gln Arg Ala Leu
            260                 265                 270

agg ctt tct ctc gcg ggt cct atc ctc tcc ctg tgc ctt ctc cct tct      864
Arg Leu Ser Leu Ala Gly Pro Ile Leu Ser Leu Cys Leu Leu Pro Ser
        275                 280                 285

ctc tga
Leu                                                                  870
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<210> 4
<211> 289
<212> PRT
<213> Homo sapiens

<400> 4

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Met Trp Asn Ala Thr Pro Ser Glu Glu Pro Gly Phe Asn Leu Thr Leu
1               5                   10                  15

Ala Asp Leu Asp Trp Asp Ala Ser Pro Gly Asn Asp Ser Leu Gly Asp
            20                  25                  30

Glu Leu Leu Gln Leu Phe Pro Ala Pro Leu Leu Ala Gly Val Thr Ala
        35                  40                  45

Thr Cys Val Ala Leu Phe Val Val Gly Ile Ala Gly Asn Leu Leu Thr
    50                  55                  60

Met Leu Val Val Ser Arg Phe Arg Glu Leu Arg Thr Thr Thr Asn Leu
65                  70                  75                  80

Tyr Leu Ser Ser Met Ala Phe Ser Asp Leu Leu Ile Phe Leu Cys Met
                85                  90                  95

Pro Leu Asp Leu Val Arg Leu Trp Gln Tyr Arg Pro Trp Asn Phe Gly
            100                 105                 110

Asp Leu Leu Cys Lys Leu Phe Gln Phe Val Ser Glu Ser Cys Thr Tyr
        115                 120                 125

Ala Thr Val Leu Thr Ile Thr Ala Leu Ser Val Glu Arg Tyr Phe Ala
    130                 135                 140

Ile Cys Phe Pro Leu Arg Ala Lys Val Val Val Thr Lys Gly Arg Val
145                 150                 155                 160

Lys Leu Val Ile Phe Val Ile Trp Ala Val Ala Phe Cys Ser Ala Gly
                165                 170                 175
```

```
Pro Ile Phe Val Leu Val Gly Val Glu His Glu Asn Gly Thr Asp Pro
            180                 185                 190

Trp Asp Thr Asn Glu Cys Arg Pro Thr Glu Phe Ala Val Arg Ser Gly
        195                 200                 205

Leu Leu Thr Val Met Val Trp Val Ser Ser Ile Phe Phe Phe Leu Pro
    210                 215                 220

Val Phe Cys Leu Thr Val Leu Tyr Ser Leu Ile Gly Arg Lys Leu Trp
225                 230                 235                 240

Arg Arg Arg Arg Gly Asp Ala Val Val Gly Ala Ser Leu Arg Asp Gln
                245                 250                 255

Asn His Lys Gln Thr Val Lys Met Leu Gly Gly Ser Gln Arg Ala Leu
            260                 265                 270

Arg Leu Ser Leu Ala Gly Pro Ile Leu Ser Leu Cys Leu Leu Pro Ser
        275                 280                 285

Leu
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<210> 5
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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (373)..(1629)
<223>

<400> 5

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cccgaggaac cacgggttct ggagctagga gccggaagct gggagtccgg aggagagcgg    180
agccccggagc ccggagcccg gggcggcgcg tctgggtctg gcgcttcccg actggacggc    240
gcgcccgctg gtcttcgcca cgcgccctcc cctgggctcg cgttcatcgg tccccgcctg    300
agacgcgccc actcctgccc ggacttccag ccccggaggc gccggacaga gccgcggact    360

ccagcgccca cc atg cgc ctc aac agc tcc gcg ccg gga acc ccg ggc acg    411
              Met Arg Leu Asn Ser Ser Ala Pro Gly Thr Pro Gly Thr
              1               5                   10

ccg gcc gcc gac ccc ttc cag cgg gcg cag gcc gga ctg gag gag gcg    459
Pro Ala Ala Asp Pro Phe Gln Arg Ala Gln Ala Gly Leu Glu Glu Ala
    15                  20                  25

ctg ctg gcc ccg ggc ttc ggc aac gct tcg ggc aac gcg tcg gag cgc    507
Leu Leu Ala Pro Gly Phe Gly Asn Ala Ser Gly Asn Ala Ser Glu Arg
30                  35                  40                  45

gtc ctg gcg gca ccc agc agc gag ctg gac gtg aac acc gac atc tac    555
Val Leu Ala Ala Pro Ser Ser Glu Leu Asp Val Asn Thr Asp Ile Tyr
                50                  55                  60
```

```
tcc aaa gtg ctg gtg acc gcc gtg tac ctg gcg ctc ttc gtg gtg ggc      603
Ser Lys Val Leu Val Thr Ala Val Tyr Leu Ala Leu Phe Val Val Gly
            65                  70                  75

acg gtg ggc aac acg gtg acg gcg ttc acg ctg gcg cgg aag aag tcg      651
Thr Val Gly Asn Thr Val Thr Ala Phe Thr Leu Ala Arg Lys Lys Ser
        80                  85                  90

ctg cag agc ctg cag agc acg gtg cat tac cac ctg ggc agc ctg gcg      699
Leu Gln Ser Leu Gln Ser Thr Val His Tyr His Leu Gly Ser Leu Ala
    95                  100                 105

ctg tcc gac ctg ctc acc ctg ctg ctg gcc atg ccc gtg gag ctg tac      747
Leu Ser Asp Leu Leu Thr Leu Leu Leu Ala Met Pro Val Glu Leu Tyr
110                 115                 120                 125

aac ttc atc tgg gtg cac cac ccc tgg gcc ttc ggc gac gcc ggc tgc      795
Asn Phe Ile Trp Val His His Pro Trp Ala Phe Gly Asp Ala Gly Cys
                130                 135                 140

cgc ggc tac tac ttc ctg cgc gac gcc tgc acc tac gcc acg gcc ctc      843
Arg Gly Tyr Tyr Phe Leu Arg Asp Ala Cys Thr Tyr Ala Thr Ala Leu
            145                 150                 155

aac gtg gcc agc ctg agt gtg gag cgc tac ctg gcc atc tgc cac ccc      891
Asn Val Ala Ser Leu Ser Val Glu Arg Tyr Leu Ala Ile Cys His Pro
        160                 165                 170

ttc aag gcc aag acc ctc atg tcc cga agc cgc acc aag aag ttc atc      939
Phe Lys Ala Lys Thr Leu Met Ser Arg Ser Arg Thr Lys Lys Phe Ile
    175                 180                 185

agc gcc atc tgg ctc gcc tcg gcc ctg ctg acg gtg cct atg ctg ttc      987
Ser Ala Ile Trp Leu Ala Ser Ala Leu Leu Thr Val Pro Met Leu Phe
190                 195                 200                 205

acc atg ggc gag cag aac cgc agc gcc gac ggc cag cac gcc ggc ggc     1035
Thr Met Gly Glu Gln Asn Arg Ser Ala Asp Gly Gln His Ala Gly Gly
                210                 215                 220

ctg gtg tgc acc ccc acc atc cac act gcc acc gtc aag gtc gtc ata     1083
Leu Val Cys Thr Pro Thr Ile His Thr Ala Thr Val Lys Val Val Ile
            225                 230                 235

cag gtc aac acc ttc atg tcc ttc ata ttc ccc atg gtg gtc atc tcg     1131
Gln Val Asn Thr Phe Met Ser Phe Ile Phe Pro Met Val Val Ile Ser
        240                 245                 250

gtc ctg aac acc atc atc gcc aac aag ctg acc gtc atg gta cgc cag     1179
Val Leu Asn Thr Ile Ile Ala Asn Lys Leu Thr Val Met Val Arg Gln
    255                 260                 265

gcg gcc gag cag ggc caa gtg tgc acg gtc ggg ggc gag cac agc aca     1227
Ala Ala Glu Gln Gly Gln Val Cys Thr Val Gly Gly Glu His Ser Thr
270                 275                 280                 285

ttc agc atg gcc atc gag cct ggc agg gtc cag gcc ctg cgg cac ggc     1275
Phe Ser Met Ala Ile Glu Pro Gly Arg Val Gln Ala Leu Arg His Gly
                290                 295                 300
```

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gtg cgc gtc cta cgt gca gtg gtc atc gcc ttt gtg gtc tgc tgg ctg      1323
Val Arg Val Leu Arg Ala Val Val Ile Ala Phe Val Val Cys Trp Leu
            305                 310                 315

ccc tac cac gtg cgg cgc ctc atg ttc tgc tac atc tcg gat gag cag      1371
Pro Tyr His Val Arg Arg Leu Met Phe Cys Tyr Ile Ser Asp Glu Gln
        320                 325                 330

tgg act ccg ttc ctc tat gac ttc tac cac tac ttc tac atg gtg acc      1419
Trp Thr Pro Phe Leu Tyr Asp Phe Tyr His Tyr Phe Tyr Met Val Thr
    335                 340                 345

aac gca ctc ttc tac gtc agc tcc acc atc aac ccc atc ctg tac aac      1467
Asn Ala Leu Phe Tyr Val Ser Ser Thr Ile Asn Pro Ile Leu Tyr Asn
350                 355                 360                 365

ctc gtc tct gcc aac ttc cgc cac atc ttc ctg gcc aca ctg gcc tgc      1515
Leu Val Ser Ala Asn Phe Arg His Ile Phe Leu Ala Thr Leu Ala Cys
                370                 375                 380

ctc tgc ccg gtg tgg cgg cgc agg agg aag agg cca gcc ttc tcg agg      1563
Leu Cys Pro Val Trp Arg Arg Arg Arg Lys Arg Pro Ala Phe Ser Arg
            385                 390                 395

aag gcc gac agc gtg tcc agc aac cac acc ctc tcc agc aat gcc acc      1611
Lys Ala Asp Ser Val Ser Ser Asn His Thr Leu Ser Ser Asn Ala Thr
        400                 405                 410

cgc gag acg ctg tac tag gctgtgcgcc ccggaacgtg tccaggagga             1659
Arg Glu Thr Leu Tyr
    415

gcctggccat gggtccttgc ccccgacaga cagagcagcc cccacccggg agccttgatg    1719
ggggtcaggc agaggccagc ctgcactgga gtctgaggcc tgggaccccc ccctcccacc    1779
ccctaaccca tgtttctcat tagtgtctcc ogggcctgtc cccaactcct ccccaccoct    1839
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gcctctaaca aggagaaatt agtgtgcggc aaaaggcagt tttctttgtt ctcagactaa    1959
tggatggttc cagagaagga aatgaaatgt gctgggtggg gccgggcctc cggcggcccg    2019
gctgctgttc ccatgtccac atctctgagg cctgcacccc ctctgtctag ctcggggagt    2079
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35 40 45

Ala Pro Ser Ser Glu Leu Asp Val Asn Thr Asp Ile Tyr Ser Lys Val
50 55 60

Leu Val Thr Ala Val Tyr Leu Ala Leu Phe Val Val Gly Thr Val Gly
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Asn Thr Val Thr Ala Phe Thr Leu Ala Arg Lys Lys Ser Leu Gln Ser
85 90 95

Leu Gln Ser Thr Val His Tyr His Leu Gly Ser Leu Ala Leu Ser Asp
100 105 110

Leu Leu Thr Leu Leu Leu Ala Met Pro Val Glu Leu Tyr Asn Phe Ile
115 120 125

Trp Val His His Pro Trp Ala Phe Gly Asp Ala Gly Cys Arg Gly Tyr
130 135 140

Tyr Phe Leu Arg Asp Ala Cys Thr Tyr Ala Thr Ala Leu Asn Val Ala
145 150 155 160

Ser Leu Ser Val Glu Arg Tyr Leu Ala Ile Cys His Pro Phe Lys Ala
165 170 175

Lys Thr Leu Met Ser Arg Ser Arg Thr Lys Lys Phe Ile Ser Ala Ile
180 185 190

Trp Leu Ala Ser Ala Leu Leu Thr Val Pro Met Leu Phe Thr Met Gly
195 200 205

Glu Gln Asn Arg Ser Ala Asp Gly Gln His Ala Gly Gly Leu Val Cys
210 215 220

Thr Pro Thr Ile His Thr Ala Thr Val Lys Val Val Ile Gln Val Asn
225 230 235 240

Thr Phe Met Ser Phe Ile Phe Pro Met Val Val Ile Ser Val Leu Asn
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Thr Ile Ile Ala Asn Lys Leu Thr Val Met Val Arg Gln Ala Ala Glu
260 265 270

Gln Gly Gln Val Cys Thr Val Gly Gly Glu His Ser Thr Phe Ser Met
275 280 285

Ala Ile Glu Pro Gly Arg Val Gln Ala Leu Arg His Gly Val Arg Val
290 295 300

Leu Arg Ala Val Val Ile Ala Phe Val Val Cys Trp Leu Pro Tyr His
305 310 315 320

Val Arg Arg Leu Met Phe Cys Tyr Ile Ser Asp Glu Gln Trp Thr Pro
325 330 335

Phe Leu Tyr Asp Phe Tyr His Tyr Phe Tyr Met Val Thr Asn Ala Leu
340 345 350

Phe Tyr Val Ser Ser Thr Ile Asn Pro Ile Leu Tyr Asn Leu Val Ser
355 360 365

Ala Asn Phe Arg His Ile Phe Leu Ala Thr Leu Ala Cys Leu Cys Pro
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<400> 51
tttaagcttg aagaccattt ttggaaaaaa aaaaaaaaaa aaaaaac 47

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<400> 56
gttagtgtac gaactggagt tcaagagact ccagttcgta cactaac 47

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<400> 58
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<210> 59
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<212> DNA
<213> Artificial

<220>
<223> An artifisially synthesized oligonucleotide sequence for hairpin siRNA

<400> 59
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<210> 60
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artifisially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 60
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<210> 61
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artifisially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 61
aaaagaaggc agttgaccaa cactctcttg aagtgttggt caactgcctt c 51

<210> 62
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artifisially synthesized oligonucleotide sequence for hairpin siRNA

<400> 62
gaaggcagtt gaccaacact tcaagagagt gttggtcaac tgccttc 47

<210> 63
<211> 51
<212> DNA
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<223> An artifisially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 63
tcccgagact gaacagagat gtgttcaaga gacacatctc tgttcagtct c 51

<210> 64
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<210> 69

<211> 51
<212> DNA
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<210> 70
<211> 51
<212> DNA
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<210> 71
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artifisially synthesized oligonucleotide sequence for hairpin siRNA

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<210> 72
<211> 51
<212> DNA
<213> Artificial

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<223> An artifisially synthesized oligonucleotide sequence for construction of siRNA expression vector.

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tcccgctggt catcttcgtc atcttcaaga gagatgacga agatgaccag c 51

<210> 73
<211> 51
<212> DNA
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<220>
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aaaagctggt catcttcgtc atctctcttg aagatgacga agatgaccag c 51

<210> 74
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artifisially synthesized oligonucleotide sequence for hairpin siRNA

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<210> 75
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<212> DNA
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<210> 76
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<220>
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<210> 78
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<212> DNA
<213> Artificial

<220>
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<400> 80
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Application Data Sheet

Application Information

Application number::	
Filing Date::	03/23/04
Application Type::	Provisional
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R??::	
Number of CD disks::	
Number of copies of CDs::	
Sequence Submission::	Yes
Computer Readable Form (CRF)?::	
Number of copies of CRF::	
Title::	METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER
Attorney Docket Number::	082368-000500US
Request for Early Publication::	No
Request for Non-Publication::	No
Suggested Drawing Figure::	11
Total Drawing Sheets::	11
Small Entity?::	No
Latin name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers One::	
Secrecy Order in Parent Appl.::	No

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City of Mailing Address::	
State or Province of mailing address::	Kanagawa
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Postal or Zip Code of mailing address::	

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City of Mailing Address::	
State or Province of mailing address::	Kanagawa
Country of mailing address::	Japan
Postal or Zip Code of mailing address::	

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Family Name::	Nakatsuru
Name Suffix::	
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State or Province of Residence::	Saitama
Country of Residence::	Japan
Street of Mailing Address::	
City of Mailing Address::	
State or Province of mailing address::	Saitama
Country of mailing address::	Japan
Postal or Zip Code of mailing address::	

Correspondence Information

Correspondence Customer Number:: 20350

Representative Information

Representative Customer Number:: 20350

Domestic Priority Information

Application:: Continuity Type:: Parent Application:: Parent Filing Date::

Foreign Priority Information

Country:: Application number:: Filing Date::

Assignee Information

Assignee Name::

Street of mailing address::

City of mailing address::

State or Province of mailing address::

Country of mailing address::

Postal or Zip Code of mailing address::

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference ONC-A0401P	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/JP2005/005613	International filing date *(day/month/year)* 18 March 2005 (18.03.2005)	Priority date *(day/month/year)* 23 March 2004 (23.03.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant ONCOTHERAPY SCIENCE, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 26 September 2006 (26.09.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Yoshiko Kuwahara e-mail: pt07@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2005/005613	18.03.2005	23.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
ONCOTHERAPY SCIENCE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
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Authorized Officer

Guarinos Viñals, E

Telephone No. +49 89 2399-7228

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2005/005613

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-30, 32-68

because:

☒ the said international application, or the said claims Nos. 11-25, 35-40 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☒ the claims, or said claims Nos. 9, 68 when concerning a reagent other than nucleic acid or antibody are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 8, 26, 27, 32, 33, 41, 42, 43, 63, 64, 65 completely and 1-7, 9-18, 28-30, 34-40, 44-62, 66-68 partially when concerning genes GHSR1b, NTSR1, FOXM1 orKOC1.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 19-25, 31 completely and 1-7, 9-18, 35-40, 44-62, 66-68 partially

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	19-25, 31, 35-38, 48-53, 58, 61
	No:	Claims	1-7, 9, 10-18, 39, 40, 44-47, 54-55, 57, 59, 60, 62, 66-68
Inventive step (IS)	Yes:	Claims	19-25, 31, 38, 58
	No:	Claims	35-37, 48-53, 61
Industrial applicability (IA)	Yes:	Claims	1-7, 9-10, 31, 44-62, 66-68
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item II
Priority

The International Searching Authority has not been able to consider the validity of the priority claim because a copy of the earlier application whose priority has been claimed was not available at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 11-25, as far as an *in vivo* method is concerned, and claims 35-40 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).
For the assessment of the present claims 10, 15 22, 28 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 9 and 68 relate to a reagent defined by reference to a desirable characteristic or property, namely its capacity to detect the expression of the KIF11 gene. The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a

result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the reagent as being a nucleic acid or an antibody (as defined in page 24, line 9 of the present application).

Claims 41 and 63 relate to a product defined by reference to a desirable characteristic or property, namely its capacity of being identified by a screening method. The claims cover all products having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for any of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for the subject-matter of claims 41 and 63.

Claim 8 relates to a non-small cell lung cancer (NSCLC) reference expression profile that comprises the expression pattern of two or more genes selected from the group consisting of KIF11, GHSR1b, NTSR1 and FOXM1. An expression profile is defined in page 23, lines 28-33 of the present application as the level of expression of these genes. The subject-matter of claim 8 is a mere presentation of information for which no search is required as specified in Rule 39.1(v) PCT. No search was therefore carried out by the International Searching Authority on the subject-matter of claim 8.

Claims relating to inventions in respect of which no international search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The EPO policy when acting as an International Preliminary Examining Authority is not to carry out a preliminary examination on matter which has not been searched. No examination will therefore be carried out for the subject-matter of claims 8, 41, 63. Examination will be carried out for the subject-matter of claims 9, 68 when the reagent is a nucleic acid or and antibody.

Re Item IV

Lack of unity of invention

The International Searching Authority has found that the present application does not meet the requirements of unity of invention. Five groups of inventions have been identified within the present application (see the International Search Report).

As the applicant has not paid any additional fee the examination will be restricted to the subject-matter for which an International Search Report has been established, i.e. the subject-matter of the first invention. Therefore only the subject-matter of claims 19-25, 31 completely and 1-7, 9-18, 35-40, 44-62, 66-68 partially (when concerning the KIF11 gene) will be examined according to Rule 68.5 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-B1-6 544 766 (BERAUD CHRISTOPHE ET AL) 8 April 2003 (2003-04-08).

D2: US-B1-6 472 521 (UHLMANN EUGEN ET AL) 29 October 2002 (2002-10-29).

D3: WO 03/030832 A (CHIRON CORPORATION; REINHARD, CHRISTOPH; WALTER, ANNETTE) 17 April 2003 (2003-04-17).

D4: WO 03/099224 A (ISIS PHARMACEUTICALS, INC; DOBIE, KENNETH, W; KOLLER, ERICH) 4 December 2003 (2003-12-04).

D5: KAISER ASTRID ET AL: "All-trans-retinoic acid-mediated growth inhibition involves inhibition of human kinesin-related protein HsEg5" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 274, no. 27, 2 July 1999 (1999-07-02), pages 18925-18931.

D6: WEIL D ET AL: "Targeting the kinesin Eg5 to monitor siRNA transfection in mammalian cells." BIOTECHNIQUES, vol. 33, no. 6, December 2002 (2002-12), pages 1244-1248.

D7: SHARP DAVID J ET AL: "The bipolar kinesin, KLP61F, cross-links microtubules within interpolar microtubule bundles of Drosophila embryonic mitotic spindles" JOURNAL OF CELL BIOLOGY, vol. 144, no. 1, 11 January 1999 (1999-01-11), pages 125-138.

D8: BLANGY A ET AL: "Phosphorylation by p34-cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 83, 29 December 1995 (1995-12-29), pages 1159-1169.

D9: HOULISTON EVELYN ET AL: "The kinesin-related protein Eg5 associates with both interphase and spindle microtubules during Xenopus early development" DEVELOPMENTAL BIOLOGY, vol. 164, no. 1, 1994, pages 147-159.

D10: US-B1-6 331 396 (SILVERMAN ROBERT H ET AL) 18 December 2001 (2001-12-18).

D11: US-B1-6 706 867 (LORENZ MATTHIAS) 16 March 2004 (2004-03-16).

D12: WO 01/31335 A (CYTOKINETICS, INC; WOOD, KENNETH, W; FINER, JEFFREY, T; BERAUD, CHRIST) 3 May 2001 (2001-05-03).

D13: YARROW J C ET AL: "Phenotypic screening of small molecule libraries by high throughput cell imaging." COMBINATORIAL CHEMISTRY AND HIGH THROUGHPUT SCREENING, vol. 6, no. 4, June 2003 (2003-06), pages 279-286.

D14: WO 01/94629 A (AVALON PHARMACEUTICALS) 13 December 2001 (2001-12-13).

Novelty (Art 33(2) PCT)

Document D1 discloses the human kinesin-related protein identified by SEQ ID NO:29 and encoded by SEQ ID NO:28 that corresponds to the KIF11 gene of the present application identified by SEQ ID NO: 2 and encoded by SEQ ID NO:1.
Claims 44, 45, 46, 47 as presently drafted embrace any dsDNA comprising the KIF11 gene, comprising at least 10 contiguous nucleotides from SEQ ID NO:1, comprising from 19 to 25 contiguous nucleotides from SEQ ID NO:1 or comprising SEQ ID NOs: 32, 33 or 34. This includes the dsDNA fragment encoding KIF11 and the vector comprising said dsDNA fragment as disclosed in D1.
The subject-matter of claims 44-47, 54-55, 57 is therefore not new in the light of D1.

For claims directed to a physical entity, non-distinctive characteristics of a particular intended use, are disregarded in determining novelty of the subject matter (see PCT International Search and Preliminary Examination Guidelines, Chapter 12, paragraph 12.05). Consequently "a composition for treating or preventing NSCLC comprising ..." is regarded as "a composition comprising ..." and "a method for identifying/screening for a compound for treating or preventing NSCLC comprising ..." is regarded as "a method for identifying/screening for a compound comprising ..." for the purpose of determining novelty of the subject matter.

Documents D2-D5 disclose a composition comprising an antisense polynucleotide against the KIF11 gene.
The subject-matter of claim 59 is therefore not new in the light of D2-D5.

Document D6 discloses a composition comprising a siRNA against the KIF11 gene.
The subject-matter of claim 60 is therefore not new in the light of D6.

Documents D7-D9 disclose a composition comprising an antibody that binds to a KIF11 polypeptide.
The subject-matter of claim 62 is therefore not new in the light of D7-D9.

Documents D10 and D11 disclose an array comprising a nucleic acid probe that binds to the KIF11 polynucleotide.
The subject-matter of claims 9, 10 and 68 is therefore not new in the light of D7-D9.

Documents D12 and D13 disclose screening methods for antagonists of the KIF11 polynucleotide/polypeptide. Furthermore D12 discloses methods where the test cells are lung cancerous cells and, among them, squamous cell and adenocarcinoma, i.e. a NSCLC cell.
The subject-matter of claims 11-18 is therefore not new in the light of D12 and D13.

Document D14 discloses SEQ ID NOs: 3028-5303 that correspond to genes that are differentially expressed in lung cancers, particularly in adenocarcinoma and squamous cell carcinoma. Both adenocarcinoma and squamous cell carcinoma belong to the non-small cell lung cancer (NSCLC). SEQ ID NO: 3571-3777 represent genes or gene sequences expressed in malignant lung samples that are not expressed at appreciable levels in non-malignant lung cells (see page 18, lines 19-22). Also disclosed in D14 are a method for diagnosing lung adenocarcinoma and squamous cell carcinoma by determining the expression of any of these genes and a method for treating lung adenocarcinoma and squamous cell carcinoma by administering an antibody that binds to any of these genes. SEQ ID NO: 3760 disclosed in D14 corresponds to the KIF11 gene of the present application identified by SEQ ID NO:1 and encoding SEQ ID NO:2.
The subject-matter of claims 1-7, 39, 40, 66, 67 is therefore not new in the light of D14.

Inventive step (Art 33(3) PCT)

D14 discloses a method for treating or preventing NSCLC by administering an antibody that reduces the amount of KIF11 polypeptide.
The difference between D14 and the subject-matter of claim 35 is that the method of claim

35 involves, instead of the use of an antibody, the use of an antisense against the KIF11 polynucleotide.
In the light of D14 the problem solved by the subject-matter of claim 35 is regarded as the provision of an alternative method for treating or preventing NSCLC, the solution being a method for treating or preventing NSCLC by administering an antisense against the KIF11 polynucleotide.
Said solution is not regarded as inventive because an antisense is a known alternative to suppress the expression of a polypeptide, i.e. to reduce the amount of KIF11 polypeptide.
Furthermore documents D2- D5 teach antisenses that are effective in suppressing the expression of the KIF11 polypeptide.
Therefore the subject-matter of claim 35 is not inventive in the light of D14 and D2-D5.

The difference between D14 and the subject-matter of claim 36 is that the method of claim 36 involves, instead of the use of an antibody, the use of a siRNA against the KIF11 polynucleotide.
In the light of D14 the problem solved by the subject-matter of claim 36 is regarded as the provision of an alternative method for treating or preventing NSCLC, the solution being a method for treating or preventing NSCLC by administering a siRNA against the KIF11 polynucleotide.
The provision of a siRNA that is effective in reducing the expression of the KIF11 polynucleotide is regarded as inventive because a person skilled in the art has no reasonable expectation of success when trying to provide such a siRNA. However an inventive step can only be acknowledged for those specific siRNAs that have the desired technical effect.
Claims 36, 48-53, 56 as drafted embrace virtually any siRNA and do therefore not meet the requirements of Art 33(3) PCT.
Claims 37 and 61 refer to SEQ ID NOs: 32, 33 and 34 but are not limited structurally (although a siRNA cannot have an infinite length). They do therefore not meet the requirements of Art 33(3) PCT.
Claims 38 and 58 that refer to the specific siRNAs and limit them structurally meet the requirements of Art 33(3) PCT.

The applicant has proven that the KIF11 polypeptide interacts with KOC1 forming a complex involved in mRNA transporting. There is no indication in the prior art neither about

the interaction of these two polypeptides nor about their role in mRNA transporting. Therefore the subject-matter of claims 19-25 and 31 is inventive.